Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Report
November/December 2013

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I. Principal Investigator: Edward P. Riley, Ph.D.

II. Title of Project: Administrative Core of the CIFASD U24 AA014811

III. Objectives/Specific Aims Goals

The primary role of the Administrative Core (AdminC) is to serve a central coordinating role for the CIFASD, ensuring all projects proceed efficiently and that results are shared across all projects. Beginning with Phase III of the CIFASD, the AdminC also includes an Educational Component to assist in disseminating our findings. The Specific Aims of the AdminC remain unchanged.

Aim 1. Provide scientific and administrative direction, leadership and oversight to the CIFASD. The PI coordinates interactions among the various projects and ensures that CIFASD investigators adhere to the goals and mission of the consortium. The AdminC also provides assistance and necessary administrative support to the Science Advisory Board (SAB) and CIFASD investigators, acting as the main liaison among the SAB, investigators and NIAAA.

Aim 2. Facilitate communication among the various projects using the CIFASD website, scheduled monthly conference calls, biannual meetings, and the preparation and distribution of annual progress reports. Additionally, an Educational Component has been added to the CIFASD, to make it similar to a P60 Comprehensive Center for Excellence. This will allow for the wider dissemination of scientific knowledge directed to the public, patient populations, policy makers, and professionals.

Aim 3. Provide assistance where necessary with data collection and ensure that data from the projects are uploaded into the Central Repository in a timely fashion, so that consortium data may be accessed by all CIFASD projects and, eventually, outside projects. The PI works with the Informatics Core to develop online interactive capacity among CIFASD investigators, and assists outside investigators with CIFASD materials.

Aim 4. Assist the SAB and CIFASD investigators in their annual evaluations of progress. In conjunction with the SAB, the AdminC establishes annual priorities and manages issues related to the allocation of resources.

Aim 5. Oversee the Developmental Component of the CIFASD, from project solicitation to selection to completion and to be responsible for the Educational Component.

IV. Methods

The AdminC is critical for facilitating collaborations required by the CIFASD. The primary goals of the AdminC are to provide an infrastructure that allows for effective leadership, an ease of communication among the various projects, a centralized resource center, a mechanism to ensure the distribution of our results, and a means to evaluate the success of the CIFASD. The AdminC provides direction and guidance to the projects. The AdminC provides a conduit of information exchange among the participants of the consortium by organizing and leading the monthly phone conferences and biannual meetings, creating progress reports, maintaining the website, and working closely with the Informatics Core. The AdminC also communicates with NIAAA about CIFASD issues. As the organizing body of the CIFASD, the AdminC has the role of advancing the major themes for research and assisting projects to meet these goals. With guidance from the PIs, the AdminC allocates resources and establishes mechanisms for distributing equipment to the various domestic and foreign sites (e.g. duties and/or bonds paid). The AdminC also ensures that procedures are standardized, data are entered and verified, and information is coordinated between sites and with NIAAA. The AdminC further coordinates with the Informatics Core to ensure that data sharing among the projects is effective. It also generates reports to all of the projects and ensures the dissemination of information to

researchers, as well as to groups and individuals with an interest in FASD. The AdminC provides ongoing critical evaluation of the progress of the CIFASD to ensure that the consortium meets its goals and is responsible for attracting new investigators via developmental projects, thereby allowing the consortium to evolve as new ideas and technologies emerge. Through the new Educational Component, the AdminC will ensure that appropriate materials are available for the public. In summary, the AdminC integrates components, coordinates shared resources, enhances communication, reviews projects, and assures CIFASD progress.

V. Accomplishments and Results

Conference Calls: The CIFASD Phase III conference calls have been held the first Wednesday of each month since the competitive renewal in the fall of 2012. Voice portions of the calls are conducted through AccuConference. WebEx links are now available on each call for the sharing of documents and for presentations. New this reporting period, the AdminC developed a schedule of WebEx presentations for these conference calls allowing the projects to provide updates on their research and receive feedback and guidance from the group. When possible, topic driven couplings of the projects are scheduled (time permitting) to assist in the facilitation of collaborations between the projects. The conference calls provide all CIFASD investigators with opportunities to discuss both their individual project's goals and those of the CIFASD as a whole. MP3 recordings of the voice portions of these calls are archived on the CIFASD.org website. Calls are moderated by Dr. Riley, or in his absence, Drs. Charness or Thomas.

Special Conference Calls: The AdminC assisted in facilitating smaller special area conference calls as needed. For example, a recent conference call was held between Drs. Charness, Foroud, Hammond and Leah Wetherill to discuss the genetic data within CIFASD and possible directions these investigators can explore in the future.

Meetings: The AdminC coordinated all elements of the CIFASD meeting at RSA in Orlando, FL in June 2013 and is in the process of planning the April 2014 face-to-face meeting to be held in Rockville, MD. Arrangements for the meeting include polling for date selection, contracting the sleeping room rates, reserving meeting space, completing travel arrangements for the PI, Scientific Director, Science Advisory Board and invited guests, and collating and distributing meeting materials. As the Consortium Coordinator, Dr. Riley researches and invites outside experts as guests to the meeting. The group has been asked for names of individuals they would like to be invited to the April meeting. Invited guests provide interesting new perspectives to CIFASD ranging from new techniques and applications that could be exciting for the group to explore and/or possibilities for new collaborations expanding CIFASD's resources.

Progress Reports: These mid-year (grant year = June 1 - May 31) reports were solicited from all CIFASD PIs and then collated by the AdminC. All progress report PDFs and PPT presentations are posted in the secure area of the CIFASD.org website. Moving forward, we will start collecting mid-year reports in November to mark the midway point of the funding period.

Project Evaluations: These mid-year progress reports are reviewed by the Science Advisory Board (SAB) which includes the AdminC PI, Scientific Director, SAB members and NIAAA staff. A conference call will be held for the SAB to evaluate the progress of each project. If needed, conference calls with the PI will be conducted to address any weak areas and troubleshooting solutions will be brainstormed. Continuing with procedure used last year, formal written project evaluations will be completed following the next face-to-face meeting in April 2014 where NIH progress reports, PPT presentations and both Central Repository and Google Doc charts will be taken into consideration while performing the reviews. As mentioned in the competitive grant renewal, these evaluations rank each project from 1 to 5 on their progress towards their aims, publications, future plans and relationship with other CIFASD projects. Following the meeting, a consensus review will be emailed to the PIs.

NIH Public Access Policy: Dr. Dale Hereld, NIAAA staff, gave a presentation to the group via WebEx during one of the monthly CIFASD conference calls to provide details regarding the NIH Public Access Policy and to illustrate the steps required to ensure all CIFASD publications are in compliance. With guidance from NIAAA, AdminC staff assembled a quick links guide and helpful hints sheet for CIFASD to reference as they complete the required steps to receive a PMCID number for their publications. The prepared resource also included detailed instructions and contacts for the journals and publishers CIFASD researchers publish in most frequently (particularly for Method C and D journals/publishers).

CIFASD.org: The AdminC updated and maintained the CIFASD.org website. Latest news and upcoming events are updated on a monthly basis and the group is reminded to forward these items to Dr. Thomas each month on the conference calls. During the last reporting period, the AdminC facilitated extensive changes to the CIFASD.org website to better communicate our findings to the general public. First, the home page was improved to be more user friendly and to highlight findings not only by the CIFASD, but the larger fetal alcohol research community. Scientific findings are presented on the site in lay language so that it is accessible to a broad audience. The site now includes more lay descriptions of each CIFASD scientific project so that our mission and methods are understandable. Finally, an Education section was added which includes videos of researchers discussing issues and findings within the field as well as the CIFASD slide set. With these modifications, the AdminC can better keep the public abreast of the current state of FASD knowledge. This resource is accessible to parents and family members, FASD advocates, educators, social workers, legal workers, and others who share an interest in improving our understanding of FASD.

CIFASD Publications: With the redesign of the website, the AdminC modified the Publication section of the website to a PubMed generated listing. This gives the public one-click access to the abstracts of our publications and the free PubMed Central full article versions as available. This supersedes the previous Google Doc system maintained by the AdminC.

Central Repository and Google Drive Docs: The Informatics Core supplies monthly reports to Dr. Riley charting the data that has been entered into the Central Repository. Spreadsheets housed within Google Drive monitoring the progress of data collection for CIFASD clinical projects are maintained by the AdminC. Projects update their completed recruitment and testing numbers on a monthly basis. During this reporting period, AdminC staff reworked the Google Docs based on recommendations from NIAAA staff to show the full 5-year trajectory of each project's aims. The modified data tables and graphs illustrate both the project's monthly progress and the goals across the project's full timeline (see the attached Addendum).

CIFASD Promotion and Recruitment: Both the PI, Dr. Riley and the Scientific Director, Dr. Charness have made several presentations promoting CIFASD in 2013. A detailed listing of their presentations can be found below in the Presentations section (X) of this report.

Dr. Riley has been invited to speak at several international engagements and his audiences have ranged from Japanese Congressmen in Tokyo, Japan to researchers and physicians in Windhoek, Nambia; both regions are exploring how to best approach the field of fetal alcohol education in their areas. He also presented on CIFASD at the ESBRA meeting in Warsaw, Poland. Stateside, he lectured to pediatric fellows at Sanford in Sioux Falls, SD and was able to introduce CIFASD to a broader audience.

Dr. Charness presented a summary of CIFASD's accomplishments to a meeting of the PASS steering committee at NIAAA and successfully engaged PASS to collaborate with the CIFASD project on 3D facial imaging. He has also spurred studies on the identification of candidate genes nominated from experiments on cellular and animal models of FASD and has organized a symposium proposal featuring CIFASD work on genetic susceptibility to FASD for the upcoming joint RSA/ISBRA meeting in Bellevue, WA in June of 2014.

These national and international presentations provide the CIFASD with exposure and also enhance the recruitment of its projects. Dr. Riley continues to extend invitations to non-CIFASD scientists to attend and present at CIFASD meetings. Outside experts bring new perspectives to the consortium and the possibilities for future collaborations or developmental projects within the CIFASD. Dr. Riley is also the current President of ISBRA and this role allows him to promote CIFASD to the international research community.

Educational Component: Importantly, the AdminC spearheaded the efforts for the CIFASD to complete the creation of a slide set to be utilized by the PIs of the Educational Component to distribute information to audiences they speak before about CIFASD. Project PIs prepared slides and the AdminC combined all slides into a fluid presentation complete with notes to be used by NOFAS in their outreach. The slide set was finalized at the June 2013 meeting and posted in the Education section of the revamped CIFASD.org website. NOFAS executives, the PIs of this Education Component, are now able to utilize this slide set in their presentations about FASD. As they give talks to groups ranging from policy makers to parents to media outlets, the work of the CIFASD consortium will be shared with new audiences. Details on their presentations and other accomplishments of this component can be found in their individual progress report which follows, including details on their newly launched monthly webinar series featuring several CIFASD investigators. Dr. Riley was the speaker for the December 2013 webinar. Funds were provided by the AdminC for Kathy Mitchell to represent CIFASD at the World Health Organization meeting held in Geneva.

Developmental Component: Two developmental projects, which started in the fall of 2012, are currently in their second year of funding. Dr. Dipak Sarkar, Rutgers, is examining circadian genes, stress and FASD. Dr. Johann Eberhart, University of Texas at Austin, is using the Zebrafish genetics model to investigate variability in FASD facial and neural phenotypes. Updates on their progress and findings follow in their individual project progress reports.

Developmental projects from Phase II of CIFASD were nearing completion in 2012 and requests for continuation of their funding were submitted via a carryover request approved in February 2013. Unfortunately, with the new funding cycle wrapping up our year at the end of May 2013 (shortening it by two months), this left little time for subcontracts to be executed and for the work to be completed. Through the received carryover funds, these previous developmental projects were given some additional time to complete their aims and following are updates on their research. Additionally, small administrative supplements and/or short-term developmental project monies were distributed to projects deemed as priorities by the SAB.

Claudia Tesche, PI. This project involves magnetoencephalographic (MEG) imaging of brain dynamics in adolescents with FASD and age- and sex-matched controls. Data are recorded during trace conditioning with aversive auditory stimuli and during performance of visually-cued finger movements. Responses are also recorded during performance of an auditory oddball task to quantify brain activation to repeated (standard) auditory stimuli. Analysis of data recorded prior to the present funding period revealed potential significant differences in brain activation between male and female participants, although data from four pairs of male subjects were unusable. During the last budget period, (August 10, 2012-May 31, 2013; grant agreement finalized on May 11, 2013), an additional two male subjects in all three tasks were recorded. Funding under this period of grant support was used to cover the MEG and structural MRI scans for these two participants and to support a course buyout for the PI in the spring of 2013. During this period, the PI performed an analysis of the combined data for the oddball task based on participant sex. These data supported significant differences in brain activation between male and female adolescents with FASD. This finding motivated re-analysis of both the conditioning and cued-movement data separately for male and for female participants. Manuscript preparation for the oddball and for the conditioning data has continued throughout the summer/fall of 2013 by the PI.

Rajesh Miranda, PI. With the carryover funds provided for use through May 2013, this project was able to perform a number of control experiments, chiefly to show that circulating miRNAs were not the products of lysis of erythrocytes, in addition to controls showing that they were not the products of leukocytes. They have resubmitted their paper to ACER with the new data and it is currently under review.

Jeff Wozniak, PI. Prior to joining CIFASD in Phase III, Dr. Wozniak provided CIFASD with access to 52 research participants with FASDs. All of these participants were seen for dysmorphology exams by Kenneth Lyons Jones, had DNA collected, and had 3D facial photographs taken by Tatiana Foroud's team. They proposed to collect neurobehavioral data and neuroimaging data on 12 of those participants and add this data to the CIFASD repository through the assistance of AdminC carryover funds. They received their completed subaward agreement at the beginning of May 2013 and, as a result, there was not sufficient time to complete the data collection prior to the 5/31/2013 end-date. The project has been started, nonetheless, and data collection for 12 participants from the initial pool of participants has been completed. The carryover funds are still needed to cover the cost of those MRI scans as well as to cover Dr. Wozniak's and Dr. Mueller's effort in collecting the data.

Alison Noble, PI. In conjunction with Peter Hammond, Alison Noble is combining her expertise in the extraction of anatomical features from 3D ultrasound images with his expertise in face shape analysis. Together this will provide an opportunity to determine if the 3D curve of the face profile is reliably segmentable as it has proven to be an effective shape discriminator in FAS and identify which facial regions reliably produce shape models good enough for face discrimination studies prenatally. Dr. Noble is currently interviewing candidates for the research fellow post and aims to start the project in January 2014.

Colleen Adnams, PI. Monies were extended to Dr. Colleen Adnams to rebuild a new subject pool in the Cape Town area so multiple CIFASD projects may continue to collect data from this rich resource. The current carryover request will include a request for the extension of these monies. Dr. Adnams is currently working on IRB approvals.

Ed Riley, PI. A small administrative supplement has been extended to Dr. Riley to allow for continued data collection for promising research projects examining children prenatally exposed to alcohol. Both studies expand the amount of information being collected from existing subjects in the San Diego site data pool, as subjects have completed the other CIFASD components.

Data collected assesses the attention and activity levels in children, and is examining the effectiveness of stimulant medication treatments in alleviating the core symptoms of Attention Deficit/Hyperactivity Disorder (ADHD) among children with and without prenatal alcohol exposure. Subjects complete the McLean Motion and Attention Test (MMAT) both on and off of their stimulant medications, for a minimum of two separate MMAT sessions. In addition to a standalone MMAT kiosk, two portable MMATs are available so that other CIFASD sites could collect this data. Secondly, children with and without prenatal alcohol exposure will undergo functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) while they complete a spatial working memory task. Additional data collection is necessary to complete an analysis examining the relationship between brain structure and function during the memory task. These studies were funded by an R01 grant that has ended; however, results indicate it would be worthwhile to continue data collection and increase the subject pool.

VI. Discussion and Significance

The AdminC has helped to facilitate the goals of the consortium and its individual projects. The AdminC has assisted in in the expansion of the consortium by funding developmental projects, requesting carryover funds, and completing administrative tasks to meet the goals of CIFASD. The PI and Scientific Director have increased the exposure of the CIFASD through their many presentations along with the expanded outreach of NOFAS in their representation for the Educational Component. Despite the budgetary cuts (competitive renewals for Phase III included 10% budget cuts and the first non-competitive renewals received an additional 7% cut), the CIFASD remains productive. The SAB continues to provide oversight and suggestions for expansions or changes in direction for the CIFASD.

VII. Interrelation with Aims of the Consortium and Other Projects

By its very nature, the AdminC interrelates with each of the CIFASD projects.

VIII. Plans for the Next Year

The AdminC will continue its charge to keep all CIFASD projects on target for their data collection and encourage the timely publication of their findings through the monthly conference calls and monitoring progress on the Google Docs. The next face-to-face meeting will convene in April 2014 and formal project evaluations will be completed by the SAB. At the start of the new funding year in June 2014, a third developmental project will be added. Dr. Foroud will begin her developmental project focusing on the identification of genetic factors that contribute to variations in response to prenatal alcohol exposure. Currently, a request for carryover of funds to complete outstanding CIFASD developmental projects is underway. As carryover is not automatic on U mechanism funds, formal requests are required each year. The nature of this process and the time it is under review at NIH coupled with the administration effort involved at both SDSU Research Foundation and the developmental project's institution truly delay the research progress of these projects. It is an unfortunate yet unavoidable cycle of the U mechanism. Timelines are further cramped by the recycling of our funding year which moved it from the August to July cycle to the current June to May funding period. With carryover funds, Dr. Riley would be able to visit several of the CIFASD sites to see firsthand their research and to gain a better grasp of any issues that need troubleshooting. Given the limited 3-month timeframe to use the carryover funds in 2013, Dr. Riley was unable to plan any such approved trips. An important charge for the AdminC moving forward will be to more closely monitor the output being generated, primarily publications and presentations, by its investment in the developmental projects.

IX. Publications

Please see the attached PDF.

X. Poster Abstract References and Presentations

2013 Edward Riley Presentations

"FASD: It's What's Behind the Face that Matters – The Effects of Prenatal Alcohol on Development, the Brain and Behavior," presented at the 54th Annual Meeting of the Japanese Society for Child and Adolescent Psychiatry, Sapporo, Japan, October 2013.

"Social, Educational and Legal Implications of FASD within the Context of Prevention or Intervention," presented at the First International Conference on the Prevention of FASD, Edmonton, Alberta, Canada, September 2013.

"The Collaborative Initiative on Fetal Alcohol Spectrum Disorders – An Overview and Update," presented at the 14th Congress of ESBRA, Warsaw, Poland, September 2013.

"What is Different About My Brain?" presented at the Living and Learning with FASD conference in Liberty Center, OH, August 2013.

"The Collaborative Initiative on Fetal Alcohol Spectrum Disorders: From the Lab to Changing Lives," presented during Pediatric Grand Rounds at Sanford USD Medical Center, Sioux Falls, SD, August 2013.

"Fetal Alcohol Spectrum Disorders: It's What's Behind the Face that Matters," lecture delivered to Pediatric Residents at Sanford Children's Hospital, Sioux Falls, SD, August 2013.

"An Introduction to FASD," presented at the FASD Children and Youth in the Justice System, an OJJDP Listening Session, Washington, DC, June 2013.

"Assessment of 30 Years of FASD Prevention in the United States," presented at the 3rd International SAF France Symposium, Paris, France, May 2013.

"Prenatal Alcohol Exposure: Effects on Brain and Behavior," lecture delivered at the Human Teratogens: Environmental Factors which Cause Birth Defects course sponsored by MassGeneral Hospital for Children and Harvard Medical School, Boston, MA, April 2013.

"Fetal Alcohol Spectrum Disorders," presented at the Training Workshop on FASD and Planning Meeting for Implementing the International Collaborative Research Project on Child Development and Prenatal Risk Factors with a Focus on FASD in the African Region, Windhoek, Namibia, April 2013.

"The Collaborative Initiative on FASD," presented at the Prenatal Alcohol Exposure and SIDS Study (PASS) site visit at Stellenbosch University, Cape Town, South Africa, April 2013.

"The Collaborative Initiative on FASD: An Update," CIFASD plenary session presentation at the 5th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, BC, March 2013.

"What Levels and Patterns of Substance Use Result in What Harm?" presented at the World Health Organization guidelines meeting, Washington, D.C., January 2013.

2013 Michael Charness Presentations

(as Scientific Director or reporting on his previous CIFASD Developmental Project findings)

Alcohol and the L1 Neural Cell Adhesion Molecule: Partners in Crime. Research Seminar Series, VA Boston Healthcare System, Boston, MA.

The L1 Neural Cell Adhesion Molecule: A Molecular Target of Alcohol Teratogenesis. Newborn Medicine Grand Rounds; Harvard Program in Neonatology. Boston Children's Hospital, Boston, MA.

The L1 Cell Adhesion Molecule is a Critical Target for Ethanol Teratogenesis. 5th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, British Columbia, Canada.

Scientific Progress of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders. Meeting of the PASS Collaborative, NIAAA, Rockville, MD.

NAPVSIPQ Blocks Ethanol Inhibition of L1 Adhesion and Ethanol Neurotoxicity. Drug Discovery and Therapy World Congress 2013; Boston, MA.

Alcohol and the L1 Neural Cell Adhesion Molecule: Opportunities for Prevention and Intervention in Fetal Alcohol Spectrum Disorders. NeuroDevNet Conference. Vancouver, British Columbia, Canada.

Alcohol and the L1 Neural Cell Adhesion Molecule. American Neurological Association, New Orleans, LA.

XI. Supplements, Training and Community

Supplements: n/a

Training: The World Health Organization utilizes a secured section of the CIFASD.org website to access CIFASD protocols for their training and data collection.

Community: The redesigned CIFASD.org site is an excellent resource the AdminC provides for the community, both to the public and other researchers. The revamped website also contains a Contact form feature and researchers have already reached out to CIFASD using this new utility for advice on neuropsychological screening tools and regarding potential involvement.

Dr. Riley continues to serve the community by presenting at many mixed audience conferences. One of particular note this year was his participation at a camp for adults with FASD and their families just outside of Toledo, OH. The CIFASD continues to present a symposium at the biennial international FASD conference in Vancouver, Canada. Its most recent plenary symposium was presented in March 2013 and the organizers were very pleased with the feedback from attendees on this session making it highly likely that the CIFASD will be asked back in 2015. This event is attended by a very broad audience including several families with individuals who have FASDs.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
In process at NIHMS	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN. Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921; NIHMSID: 538821.
In process at NIHMS	Swartz ME, Wells MB, Griffin M, McCarthy N, Lovely CB, McGurk P, Rozacky J, Eberhart JK. A Screen of Zebrafish Mutants Identifies Ethanol-Sensitive Genetic Loci. Alcohol Clin Exp Res. 2013 Oct 24;PubMed PMID: 24164477; NIHMSID: 527375.
Complete	Rachdaoui N, Sarkar DK. Effects of alcohol on the endocrine system. Endocrinol Metab Clin North Am. 2013 Sep;42(3):593-615. PubMed PMID: 24011889; PubMed Central PMCID: PMC3767933.
Complete	Eberhart JK, Harris RA. Understanding variability in ethanol teratogenicity. Proc Natl Acad Sci U S A. 2013 Apr 2;110(14):5285-6. PubMed PMID: 23513226; PubMed Central PMCID: PMC3619315.
Complete	O'Brien JW, Norman AL, Fryer SL, Tapert SF, Paulus MP, Jones KL, Riley EP, Mattson SN. Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure. Alcohol Clin Exp Res. 2013 Apr;37(4):644-54. PubMed PMID: 23094678; PubMed Central PMCID: PMC3771541.

Ukraine Progress 2012-2017		
Ukraine Aims	**Goal**	**Actual**
Recruitment Exposed	60	62
Recruitment Comparison	60	50
2 YR Old Collections	240	6
3.5-4.5 YR Neurobehavior & Collections	240	19
Date of Update:	12/3/2013	# Fields

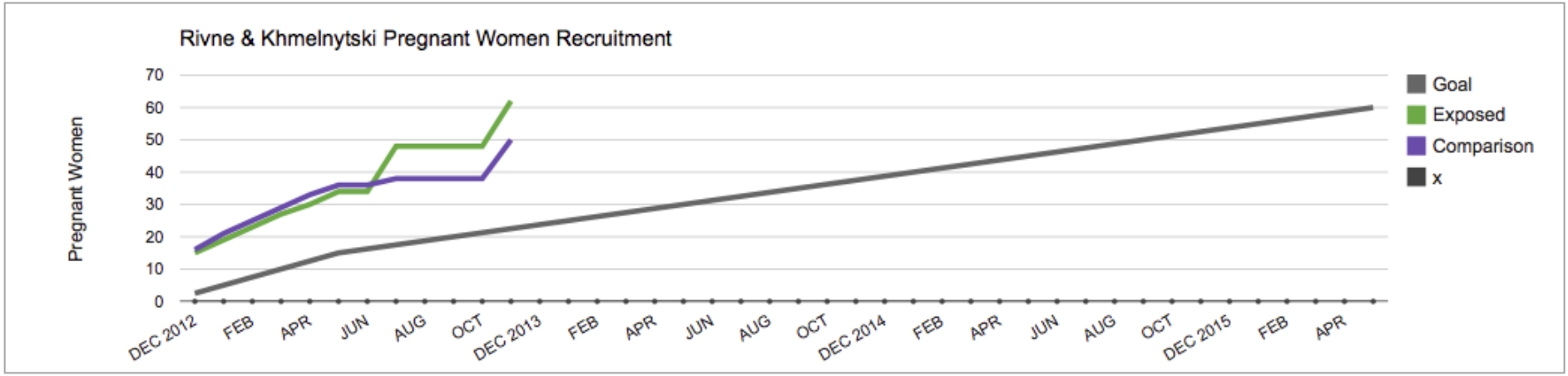
Phase III Goals (Cumulative)	Thru/By Date	**Recruitment Exposed**	**Recruitment Comparison**	**2 YR Old Collections**	**3.5-4.5 YR Neurobehavior & Collections**
Start YR1	8/10/2012	0	0	0	0
End YR1	5/31/2013	15	15	10	10
End YR2	5/31/2014	30	30	80	80
End YR3	5/31/2015	45	45	160	160
Data Collection End YR4	5/31/2016	60	60	240	240

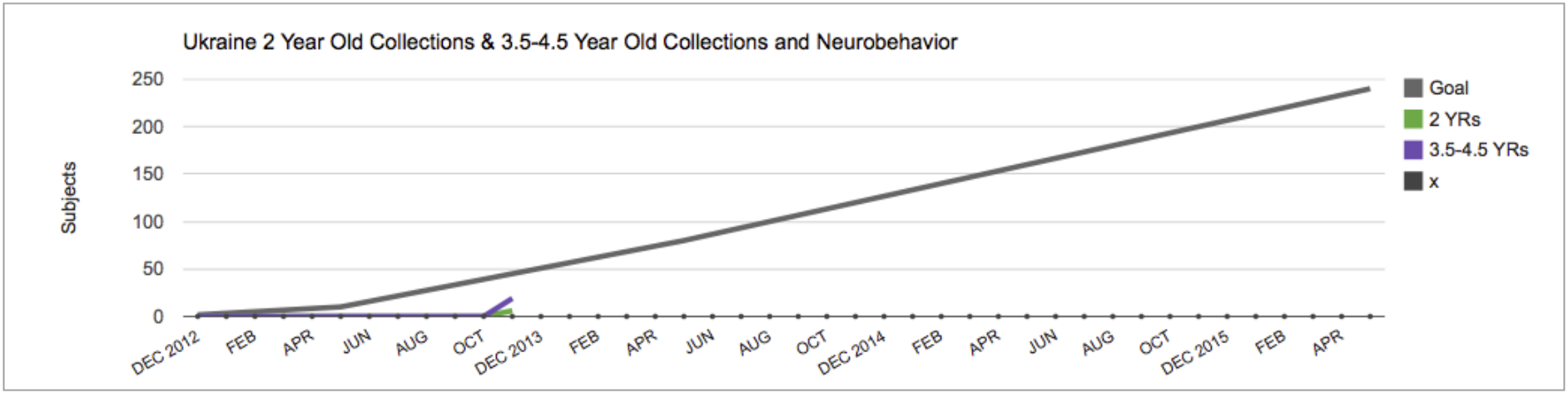
Project PI enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific.

Recruitment update justification: Due to the logistics of carrying in all new equipment, testing materials, and supplies, and the extensive training required on the new testing battery, the proposed timeline for recruitment was revised to concentrate on recruiting new pregnant women ahead of schedule, and follow as of July 1, 2013 with the preschool testing battery. This will allow for a larger pool of subjects for the child evaluations at 2 and 3.5 years.

Ukraine Progress through November 2013

X in the keys that follow is to allow for a line to run along the x-axis indicating a hash mark (dot) per month.





3D Imaging and Saliva Collection Progress 2012-2017		
3D Images	**Goal**	**Actual**
SDSU	264	93
UMN	264	101
Emory	264	56
USC	88	21
Ukraine	315	5
S. Africa PASS (Odendaal)	1080	1145
S. Africa (Jacobsons)	220	294
TOTAL	2495	1715
Saliva Samples	**Goal**	**Actual**
SDSU	264	86
UMN	264	102
Emory	264	117
USC	88	35
TOTAL	880	340
Date of Update:	12/2/2013	# Fields

3D Imaging 2012-2017

Phase III Goals Cumulative

	Thru/By Date	SDSU	UMN	Emory	USC	Ukraine	S. Africa PASS (Odendaal)	S. Africa (Jacobsons)
Start YR1	8/10/2012	0	0	0	0	0	0	0
End YR1	5/31/2013	30	30	30	10	10	900	0
End YR2	5/31/2014	96	96	96	32	95	1080	220
End YR3	5/31/2015	162	162	162	54	205	1080	220
End YR4	5/31/2016	228	228	228	76	315	1080	220
End YR5	5/31/2017	264	264	264	88	315	1080	220

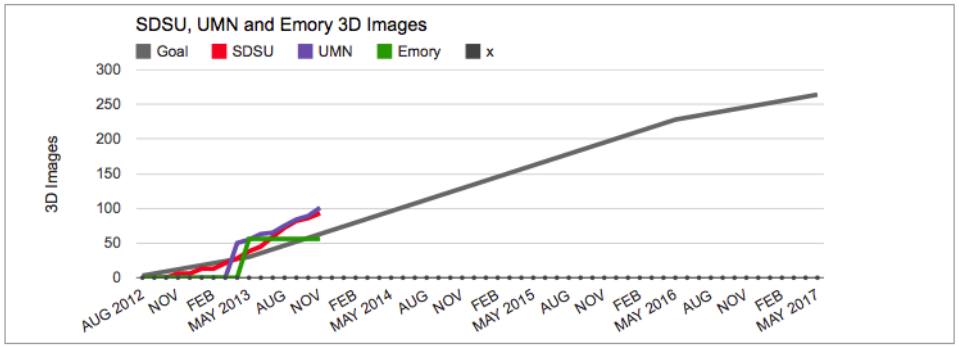
Saliva 2012-2017 (Phase III Goals Cumulative)

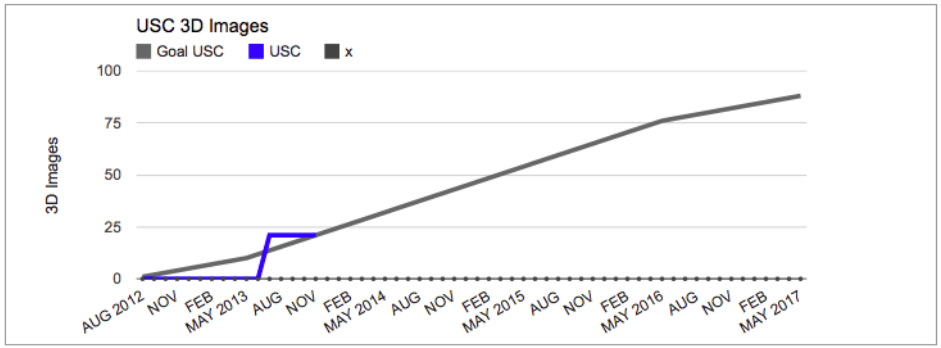
	Thru/By Date	SDSU	UMN	Emory	USC
Start YR1	8/10/2012	0	0	0	0
End YR1	5/31/2013	30	30	30	10
End YR2	5/31/2014	96	96	96	32
End YR3	5/31/2015	162	162	162	54
End YR4	5/31/2016	228	228	228	76
End YR5	5/31/2017	264	264	264	88

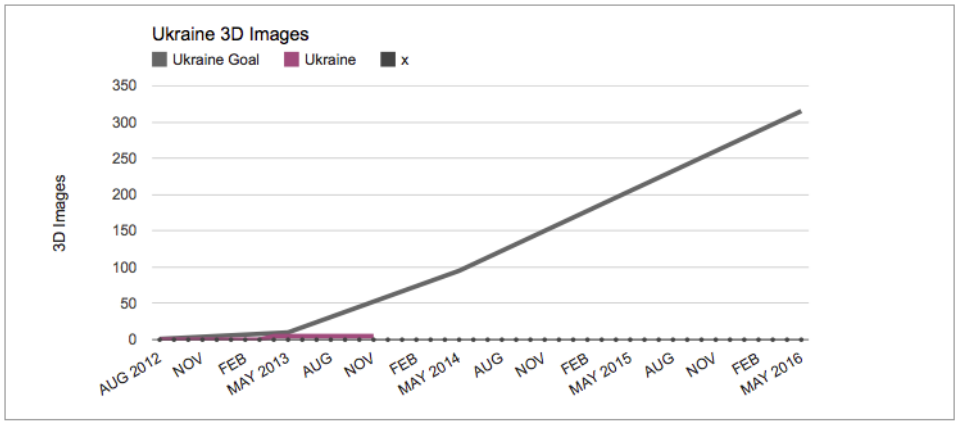
Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific.

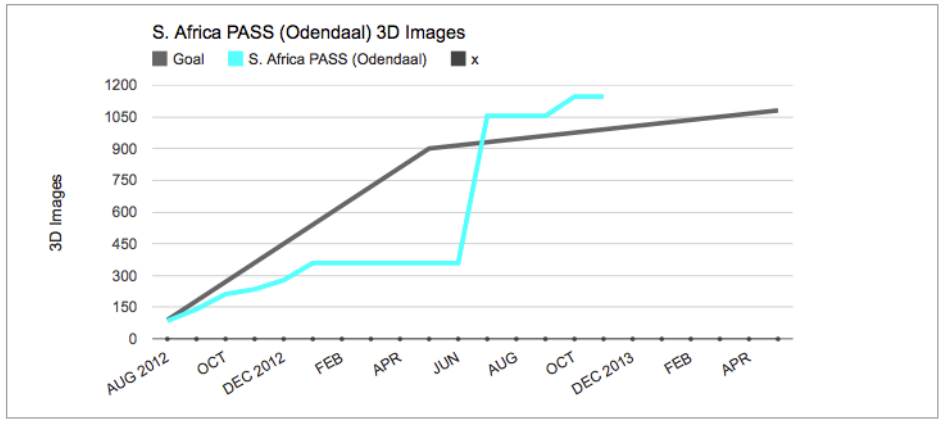
Explanation of modification to grant goals: Goals in this table reflect numbers provided by site PIs. Actual numbers reflect those for which an image has been received. Due to slow and inadequate internet service prohibiting electronic upload of files, two sites mail images on drives in batches therefore, monthly totals may not reflect the date the image was collected. Two sites do not have cameras and therefore schedule collection of images to coordinate with a clinic visit from Dr. Ken Jones. These sites will not produce continuous data month to month.

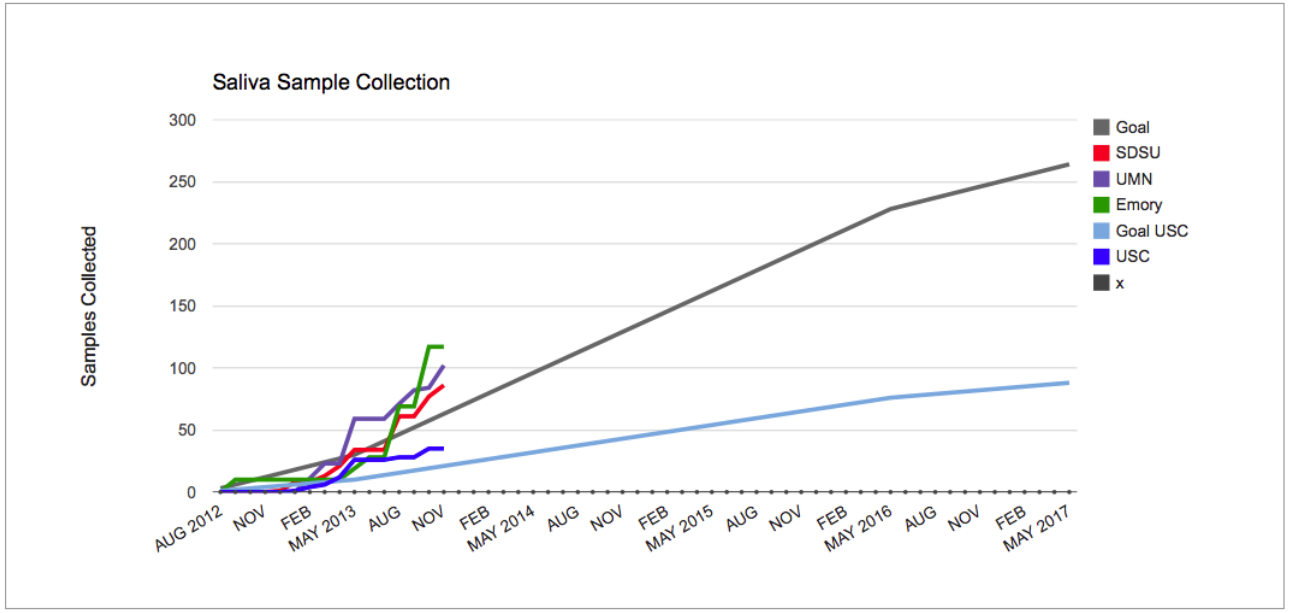
3D Images and Saliva Sample Collection through November 2013 [294 3D Images at the South Africa (Jacobsons' site) now collected.]











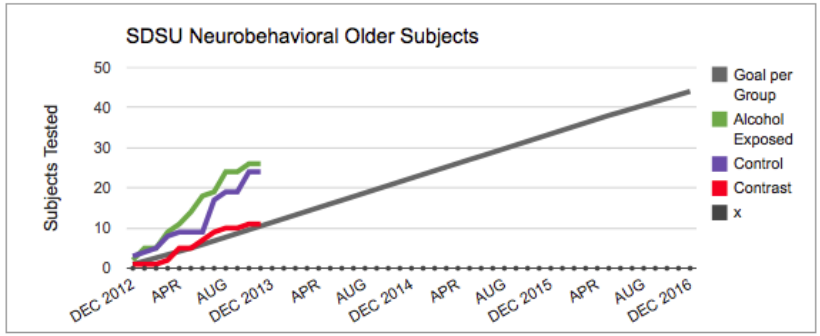
Neurobehavioral Progress 2012-2017				
Site	**Goal Per Group**	**Alcohol-Exposed**	**Control**	**Contrast**
SDSU Old	44	26	24	11
SDSU Young	44	8	12	5
UMN Old	44	28	22	17
UMN Young	44	6	12	2
Emory Old	44	10	14	6
Emory Young	44	13	5	7
USC Old	44	15	10	n/a
	All Groups & Ages	**Alcohol-Exposed**	**Control**	**Contrast**
Tested Total	253	106	99	48
Goal Total	880	308	308	264
Date of Update:	12/2/2013	# Fields	Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific; #s are cumulative.	

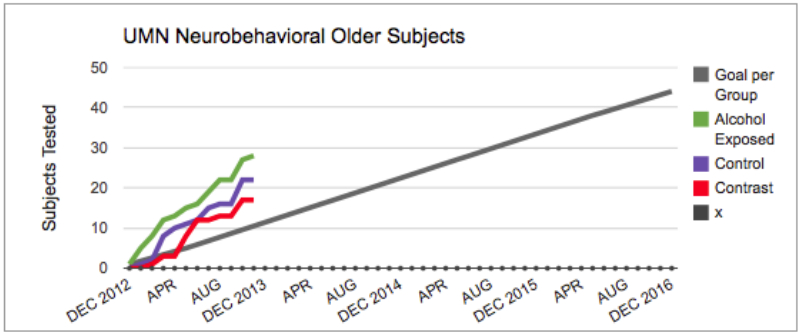
Site		SDSU						UMN						Emory						USC	
Goals (Cumulative)		Old			Young			Old			Young			Old			Young			Old	
Data Collection Subgroups	Thru/By Date	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON
Start YR1	8/10/2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
End YR1	5/31/2013	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
End YR2	5/31/2014	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
End YR3	5/31/2015	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
End YR4	5/31/2016	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
Data Collection End YR5	12/1/2016	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44

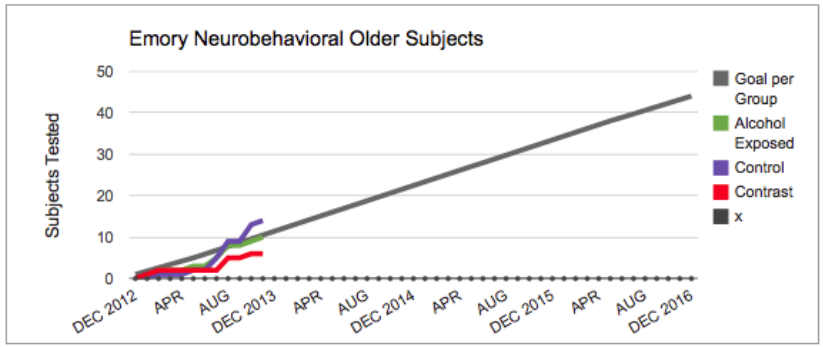
Total group goals across all sites for the older age study = 176 alcohol exposed, 176 control and 132 contrast for a total of 484.
Total group goals across all sites for the younger age study = 132 alcohol exposed, 132 control and 132 contrast for a total of 396. Both studies total = 880.

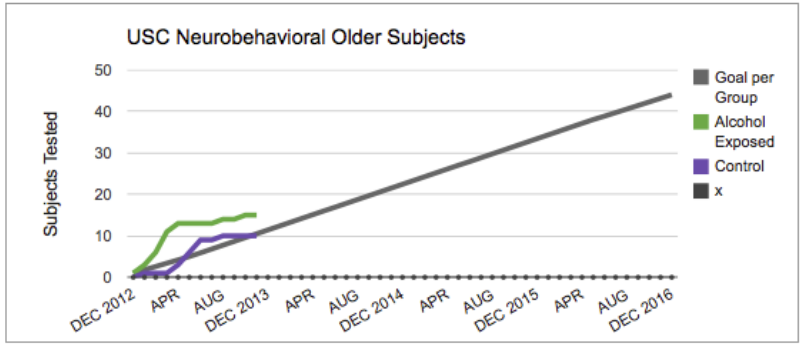
Explanation of Data Collection Goal Modification: Original proposal of 9 per year (5) per group (20) for a total of 900 (Grant Table 3) was incorrect; numbers should have followed the proposed timeline table in the grant. Overall numbers reduced by 2% to account for shortened first year and budget cuts.

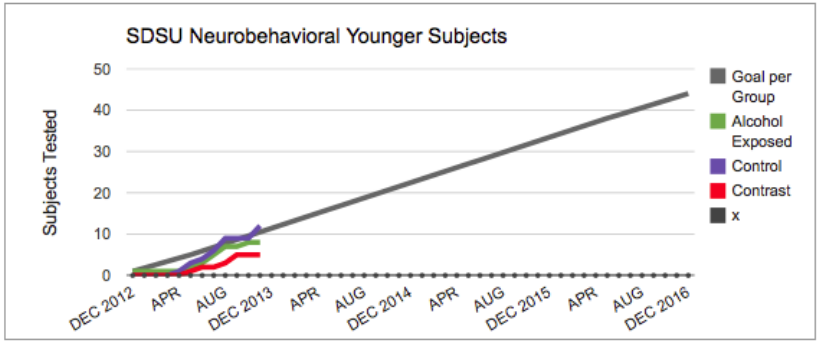
Neurobehavioral Subjects through November 2013

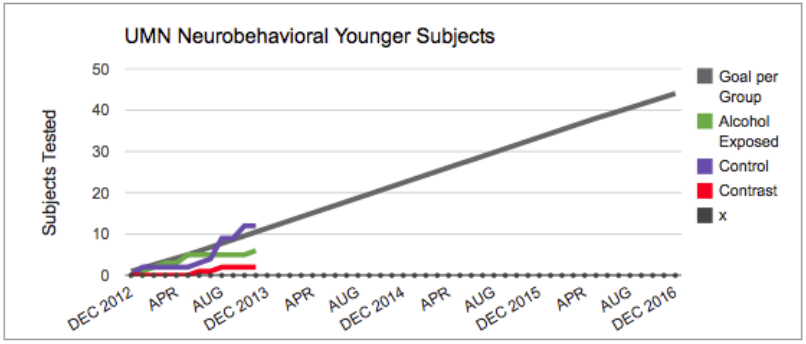


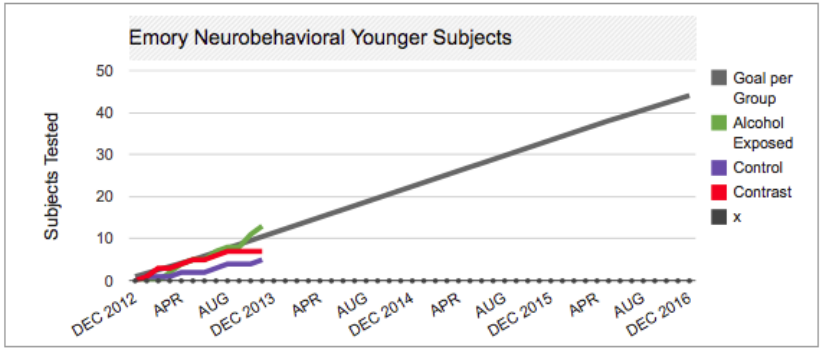












Neuroimaging Progress 2012 - 2017				
Site	**Time 1 Goal**	**Time 1 Scans**	**Time 2 Goal**	**Time 2 Scans**
SDSU AE	30	21	30	0
SDSU CON	30	9	30	0
UMN AE	30	28	30	0
UMN CON	30	21	30	0
Emory AE	30	19	30	0
Emory CON	30	13	30	0
USC AE	30	21	30	0
USC CON	30	14	30	0
TOTAL	240	146	240	0
240 goal at each scan time = 120 AE and 120 CON				
Date of Update:	12/3/2013	# Fields		

Site		SDSU				UMN				Emory				USC			
Goals (Cumulative)		Time 1		Time 2		Time 1		Time 2		Time 1		Time 2		Time 1		Time 2	
Data Collection Subgroups	Thru/By Date	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON
Start YR1	8/10/2012	0	0			0	0			0	0			0	0		
End YR1	5/31/2013	10	10			10	10			10	10			10	10		
End YR2	5/31/2014	20	20			20	20			20	20			20	20		
End YR3	5/31/2015	30	30	10	10	30	30	10	10	30	30	10	10	30	30	10	10
End YR4	5/31/2016			20	20			20	20			20	20			20	20
Data Collection End YR5	5/31/2017			30	30			30	30			30	30			30	30

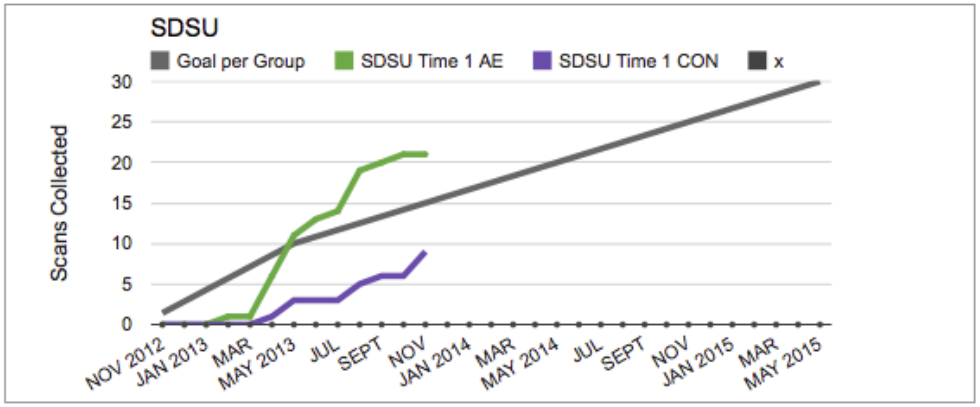
Each site will scan 30 AE and 30 CON two times for a total of 120 scan per site. Overall project unique subject goals are 120 AE and 120 CON for a total of 240 unique participants, therefore, matching the proposed grant totals.

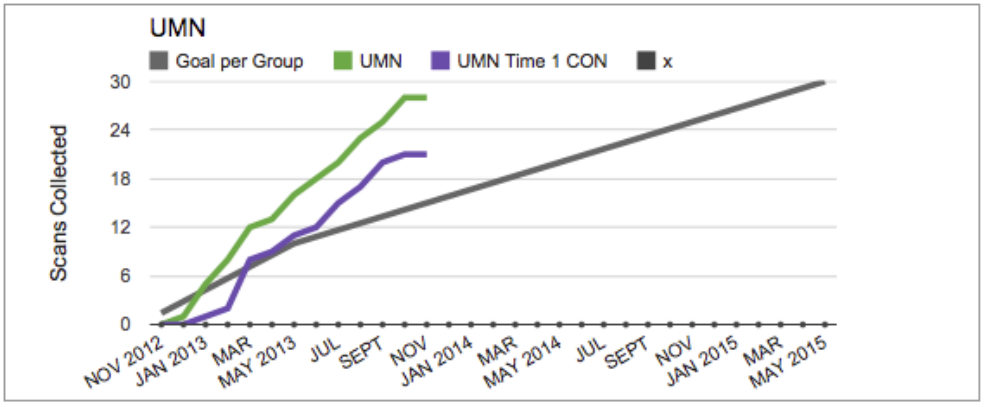
All Sites T1	**AE**	89	**CON**	57	**All Sites T2**	**AE**	0	**CON**	0	Time 2 Scan collection begins June 2014.

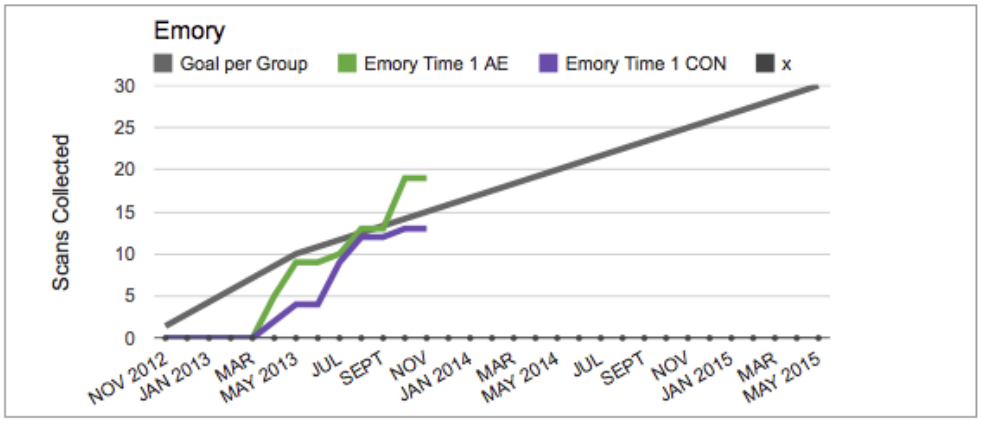
120 goal per group T1 AE, T1 CON, T2 AE, and T2 CON

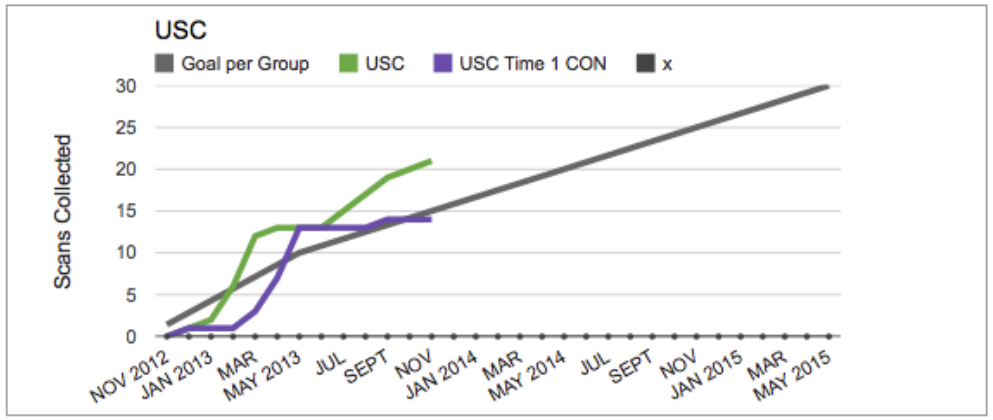
Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific; #s are cumulative.

Neuroimaging through November 2013









Percentages to Overall Phase III Goals

Neuroimaging

Percent to Overall Goals	SDSU	UMN	Emory	USC
Time 1 AE	70%	93%	63%	70%
Time 1 CON	30%	70%	43%	47%
Time 2 AE	0%	0%	0%	0%
Time 2 CON	0%	0%	0%	0%

Ukraine

Ukraine Aims	% to Goal
Recruitment Exposed	103%
Recruitment Comparison	83%
2 YR Old Collections	3%
3.5-4.5 YR Neurobehavior & Collections	8%

Neurobehavioral

Old Percent to Goal	SDSU	UMN	Emory	USC
Alcohol-Exposed	59%	64%	23%	34%
Control	55%	50%	32%	23%
Contrast	25%	39%	14%	n/a
Young Percent to Goal	**SDSU**	**UMN**	**Emory**	
Alcohol-Exposed	18%	14%	30%	
Control	27%	27%	11%	
Contrast	11%	5%	16%	

3D Imaging & Saliva

3D Images	% to Goal
SDSU	35%
UMN	38%
Emory	21%
USC	24%
Ukraine	2%
S. Africa PASS (Odendaal)	106%
S. Africa (Jacobsons)	134%

Saliva Samples	% to Goal
SDSU	33%
UMN	39%
Emory	44%
USC	40%

Total NB Tested	
SDSU	86
UMN	87
Emory	55
USC	25
	253

November 11, 2013
Individual mid-year CIFASD Progress Report

I. Principal Investigator
Co-PI's: Thomas Donaldson and Kathleen T Mitchell, MHS

II. Title of Project
Educational Component of the Administrative Core of CIFASD

III. Objectives/Specific Aims/Goals
The primary goal of the NOFAS component is to present both published and unpublished information and data from CIFASD research projects to diverse audiences both nationally and internationally through in-person presentations and multi-media channels. Audiences reached include: medical and allied health professionals and students; educators and school administrators; scientists and researchers; and lay audiences including the public at-large.

NOFAS also communicates CIFASD published findings to the media and, upon request, to national and state policymakers and public officials.

The major goals and objectives of the educational component have not changed since the previous report

IV. Methods
NOFAS has incorporated CIFASD information and data as significant content in all presentations and trainings. Additionally, NOFAS promotes CIFASD findings and news through the NOFAS Weekly Roundup, an online newsletter, various social media platforms including Facebook, Twitter and YouTube and other channels such as nofas.org.

V. Accomplishments and Results
See Supplements, Training, and Community below.

VI. Discussion and Significance
NOFAS encourages public health officials and professionals to incorporate published findings in health advisories, project development and implementation, and health promotion initiatives.

VII. Interrelation with Aims of the Consortium and Other Projects
NOFAS participates in consortium telephone conferences and meetings and interacts with consortium members throughout the year offering numerous channels for CIFASD scientists to reach both the FAS and non-FAS professional and nonprofessional communities. NOFAS assists in bringing the often complex objectives and characteristics of the initiative's basic, biomedical and clinical research to diverse audiences, including individuals living with FAS and their families, in a clear and comprehensible manner, and helps to promote the rationale for CIFASD's important research.

VIII. Plans for the Next Year
NOFAS plans to continue presenting and promoting CIFASD information and data to its audiences, and plans to add the following components:

- Monthly one-hour webinars that will feature CIFASD researchers;
- Enhanced presence on nofas.org and NOFAS social media, including videotaped interviews with CIFASD scientists;
- Enhanced media outreach.

IX. Publications
N/A

X. Poster Abstract References and Presentations
N/A

XI. Supplements, Training, and Community
During the report period, NOFAS has conducted the following presentations:

April 2013
- Presented a workshop for the Missouri Drug Court Annual Conference.
- Presented plenary for the Indiana Perinatal Task Force conference.
- Facilitated two-day FASD knowledge and skills training for healthcare professionals from the White Earth Reservation, MN.

May 2013:
- Presented a lunchtime keynote for the Alaska RTC at their FASD Conference in Anchorage, AK.
- Provided a three-hour workshop for healthcare providers in Richmond, VA for the Virginia Department of Health and Human Services.

June 2013
- Provided a Grand Rounds for the behavioral psychologists and residents of the Johns Hopkins Kennedy Krieger Institute in Baltimore, MD.
- Presented to 25 NOFAS affiliates at the 2013 NOFAS Affiliate Network Summit.
- Upon request, presented published findings to the staff of Senators Tim Johnson, Lisa Murkowski and Lamar Alexander.

July 2013
- Provided a keynote at a community FASD conference for a group of health care providers, social workers, and foster care staff in Omaha, Nebraska.
- Provided keynote on FASD and two workshops on FASD and Empowering Women for the Minnesota American Indian Substance Abuse Conference held at the Fond du Lac reservation.
- Upon request, presented published findings to the staff of Senators Mark Begich, Patty Murray, Barbara Boxer, Mary Landrieu, Ron Johnson, Tom Coburn, Sherrod Brown and Tom Harkin.

August 2013
- Provided a workshop for the staff from Crossroads Treatment Center, a large addiction center in Fairfax County, Virginia.
- Presented to the Caron Treatment Centers clinical leadership team to discuss addressing FASD assessment and diagnosis and modification of care within their national network.
- Upon Request, presented published findings to the staff of the House of Representatives Energy and Commerce Committee, Health Subcommittee, and Representative Eric Cantor.

September 2013
- Facilitated Webinar, “Creating Resiliency in Families Living with FASD” for the Arc Healthmeet.
- Conducted an interview for the international *Living with FASD 2013 Summit***.**

- Presented at the University of California, San Diego event to commemorate the work of Dr. Ken Jones and the 40th year milestone of the first FAS paper in U.S. medical literature.
- Presented *The NOFAS Experience: 25 Years of Policy, Media and Prevention* at the 40th Anniversary of FAS conference in Atlantic City, New Jersey.
- Presented two oral presentations and one panel at the First International Prevention of FASD Conference in Edmonton, Alberta, Canada.
- Presented at "Fetal Alcohol Spectrum Disorders: Developing Practical Supports 2013 Summit" in Little Rock, Arkansas.
- Upon request, presented published findings to the staff of Representatives Don Young, Frank Pallone and Jim Moran, Senator Dick Durbin, Ohio Governor John Kasich, and state lawmakers in Texas, Virginia, Maryland and Illinois.

October 2013
- Presented to a delegation of visiting government leaders, public health officials, and physicians visiting Washington, D.C. from Guangzhou Municipality in China.
- Presented two Grand Rounds to the medical staff at the Dayton Children's Hospital in Dayton, Ohio.
- Presented to the Georgetown University pre-med disease prevention and health promotion class.
- Presented to the staff from the Father McKenna Center, the only daytime, drop-in homeless center for men in Washington, D.C. The center provides alcohol treatment and other services.

November 2013
- Presented a plenary for the Virginia Juvenile Justice Conference, Charlotte, VA.

Future Training/Presentations
- Planning monthly webinars, to be held on the 3rd Wednesday of each month that will feature CIFASD Researchers. November 20, 2013 to October 15, 2014.
- Kathy Mitchell serving on the International Expert Committee to plan the UBC 6th International Conference on FASD (March 4-7 2014).
- Abstracts were submitted and accepted for a panel presentation at the UBC FASD Conference on Adolescents and Adults (April 9-12, 2013).

I. Principal Investigator Johann K. Eberhart

II. Title of Project Genetic Approaches to Understand Variability in FASD Facial and Neural Phenotypes

III. Objectives/Specific Aims/Goals
The major goals of this award are: 1) characterize ethanol-sensitive mutants from a forward genetic screen and 2) Screen community resources to identify ethanol-sensitive loci involved in facial or neural development. These goals have not changed.

IV. Methods
For Aim 1, we have generated an AB/Tu hybrid mapping background for all the forward genetic mutant lines. AB is the genetic background in which the mutagenesis took place and Tu is the reference genome that we will use for mapping. We are currently obtaining carrier fish from these backgrounds with which to perform our mapping.

For Aim 2, we screened 20 mutants obtained from ZIRC for sensitivity to ethanol teratogenesis. Embryos were treated with 1% ethanol from 6 hours post fertilization onward. Levels of apoptosis were evaluated at 24 hpf. At 5 days post fertilization, the craniofacial skeleton, peripheral axon projections and sensory neurons of neuromasts were examined. Fish were genotyped to determine if there were phenotype/genotype correlations.

V. Accomplishments and Results
We have completed our initial screen of community resources in Aim 2 and these results have recently been published in ACER. Five of the twenty loci interacted with ethanol. Notable among these was that *vangl2*, involved in planar cell polarity, interacted synergistically with ethanol. Untreated *vangl2* mutants had normal craniofacial morphology while severe midfacial defects including synophthalmia and narrowing of the palatal skeleton were found in all ethanol-treated mutants and a low percentage of heterozygotes. The cell cycle gene, *plk1*, also interacted strongly with ethanol. Untreated mutants had slightly elevated levels of apoptosis and loss of ventral craniofacial elements. Exposure to ethanol resulted in extensive apoptosis along with loss of neural tissue and the entire craniofacial skeleton. Phenotypes of *hinfp, mars* and *foxi1* mutants were also exacerbated by ethanol.

Based on the strength of the *vangl2*/ethanol interaction, we explored if there was evidence for interactions between ethanol and members of the planar cell polarity pathway in humans. In collaboration with Dr. Foroud, we have found support for interactions between ethanol and several planar cell polarity pathway members, including *VANGL1, CELSR2* and *FAT4*. Of these, the SNP in *FAT4* results in an Alanine to Valine missense mutation. In the absence of alcohol exposure, this minor allele associates with reduced lower facial depth. While alcohol exposed children homozygous for the major allele also have reduced facial depth, alcohol-exposed children with the minor allele have a facial depth similar to that of unexposed children homozygous for the major allele. This intriguing finding may suggest that the minor allele is more resistant to the effects of ethanol. Consistent with the model, this same mutation has been associated with a decreased risk of alcohol-associated cancer.

There are SNPs that interact with ethanol in the 3' UTR of other planar cell polarity members, including *CELSR2*. Because the 3' UTR is often associated with mRNA stability or translation, we are currently fusing 3' UTR of zebrafish *celsr2* as well as the major and minor alleles of the 3' UTR of human *CELSR2* to Kaede in order to determine the physiological relevance of these SNPs on protein levels.

VI. Discussion and Significance
Our findings provide direct evidence of the genes that regulate sensitivity to ethanol teratogenesis. The zebrafish provides a model system in which to rapidly identify these susceptibility loci. Through continued interactions with other CIFASD members, we will be able to leverage these results to identify those gene-ethanol interactions most likely to mediate sensitivity to FASD. Once identified, the zebrafish model system also provides an excellent system in which to determine the mechanism of gene-ethanol interactions as we have done in our recent Development publication.

VII. Interrelation with Aims of the Consortium and Other Projects
We have worked closely with Dr. Foroud to determine the conservation of gene-ethanol interactions between zebrafish and human. Additionally, we are working with Dr. Sulik to determine if candidate genes (e.g. *lrp2*) interact with ethanol.

VIII. Plans for the Next Year
In Aim 1, we anticipate sequencing 2 to 3 of the forward genetic mutants in Aim 1. We will use the TACC supercomputer available to us at UT Austin to align the sequence reads to the reference genome and scan for potential mutagenic lesions. As mutagenic lesions are identified we will determine if there is evidence for homologous gene-ethanol interactions in human and further characterize the etiology of the defects.

For Aim 2, we will continue our analyses of the planar cell polarity pathway and ethanol. We are currently obtaining mutants for the homologs of pathway members implicated in Dr. Foroud's SNP-ethanol dataset. We will screen these mutants for ethanol sensitivity. Our analyses will include characterizations of dosage and timing of ethanol sensitivity. We will further characterize those gene-ethanol interactions that show the greatest level of synergy.

We will determine if the planar cell polarity pathway SNP-ethanol interactions that we have identified in Dr. Foroud's dataset have functional significance. Because the amino acid affected by the *FAT4* SNP is not conserved in zebrafish, we plan to generate an *in vitro* model system, most likely in HEK cells, to test the functional role of the *FAT4* minor allele in stabilizing the planar cell polarity protein complex. We are also actively pursuing genetic approaches to determine if SNPs in the 3'UTR of planar cell polarity pathway genes regulate ethanol sensitivity. We are constructing transgenic zebrafish expressing fluorescent proteins, such as Kaede, fused to variants of the human 3' UTR. Our goal is to determine if non-coding variants regulate protein levels.

Collectively, we plan that our results will allow for the submission of two R01 proposals. The first will be submitted in late November 2013, and because I have continuous submission status will be reviewed in the 3rd cycle. In this grant, we are proposing continuing our screening of zebrafish mutants for craniofacial and neural defects. We anticipate submission of the second grant in 2014. The proposal will focus on the involvement of the planar cell polarity pathway in ethanol teratogenesis. In this grant, we will propose analyses of human polymorphisms on the function of the planar cell polarity pathway. We will focus on the missense mutation in FAT4 as well as polymorphisms in the 3' UTR of *CELSR2*.

IX. Publications
See attached list.

X. Poster Abstract References and Presentations

Invited seminars:

1). Stower's Institute for Medical Research, Kansas City, MO, May 2013, "Generating variation in craniofacial disease"

2). UNC-Chapel Hill, Chapel Hill, NC, April 2013 "What's behind the screen: using zebrafish to identify gene/ethanol interactions"

3). North Carolina Central University, Durham, NC, April 2013 "What's behind the screen: using zebrafish to identify gene/ethanol interactions"

Platform presentation:

8th European Zebrafish Meeting, Barcelona, Spain, July 2013 "What's behind the screen: using zebrafish to identify gene/ethanol interactions"

XI. Supplements, Training, and Community

Supplements:

None

Presentations to communities of interest:

UT Austin SAGE (continuing education group for senior citizens), Austin, TX, Oct 2013 "Fetal Alcohol Spectrum Disorders"

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Non-compliant	Swartz ME, Wells MB, Griffin M, McCarthy N, Lovely CB, McGurk P, Rozacky J, Eberhart JK. A Screen of Zebrafish Mutants Identifies Ethanol-Sensitive Genetic Loci. Alcohol Clin Exp Res. 2013 Oct 24;PubMed PMID: 24164477.
In process at NIHMS	McCarthy N, Wetherill L, Lovely CB, Swartz ME, Foroud TM, Eberhart JK. Pdgfra protects against ethanol-induced craniofacial defects in a zebrafish model of FASD. Development. 2013 Aug;140(15):3254-65. PubMed PMID: 23861062.

I. Principal Investigator: Sarkar, Dipak K.

II. Title of Project: **Circadian Genes, Stress Axis and Fetal Alcohol Spectrum Disorder**

III. Objectives/Specific Aims/Goals

What are the major goals of the project? The major goal of this project is to measure Per genes methylation and gene expression as well as plasma cortisol levels using the DNA and plasma samples of FASD and non-FASD patient children from the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) using a structured protocol for diagnosis of FAS using the cardinal facial and growth features collected from Ukrainian population by Christina Chambers and/or South African population by Sara Mattson. Once available, we will compare gene and hormone data of subjects classified as FAS, deferred (some characteristic features of FAS), or no FAS. Individuals with FAS, deferred and no FAS will be longitudinally assessed, with genotypes and phenotypical characterization for a period of 3 years. The questions to be addressed here are: Do methylation and expression levels of *Per* genes correlate with the stress abnormality (high cortisol)? Can these methylation sites and patterns be used as biomarkers that predict future endophenotypes of stress abnormalities (e.g., anxiety, alcohol binging or immune abnormalities)? Furthermore, if *Per* genes hypermethylation is involved in stress abnormalities, whether this is manifested in patients with FASD. These studies will be crucial in determining the stability of identified epigenetic signature of FASD patients and whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

Have the major goals changed since the initial competing award or previous report?
We have not received any blood DNA or plasma samples from Ukrainian or South African FASD and non-FASD patient children cohort yet. However, I have learned that Dr. Christina Chamber currently processing samples from Ukrainian patients cohort and we will be receiving the DNA and plasma samples soon. I am looking forward to receive these samples and conducting the proposed research. While waiting for the blood DNA and plasma samples, I was able to obtain salivary DNA samples from FAS, alcohol-exposed and control children for a study conducted by Dr. Tatiana Foroud at University of Indiana. We have used these samples to generate some exciting preliminary data. I have also worked with Drs. Foroud and Elizabeth R. Sowell at USC to establish a salivary collection method for IRB approval for future measurements of gonadal and stress hormones from salivary samples of FASD and non-FASD patient children collected at different sites by the CIFASD researchers. I am also looking forward to receive these samples. Hence, my current goal is expanded to determine Per genes and stress hormone regulatory POMC gene methylation and gene expression as well as plasma cortisol/testosterone levels using the blood and salivary DNA and plasma samples of FASD and non-FASD patient children from CIFASD to determine whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

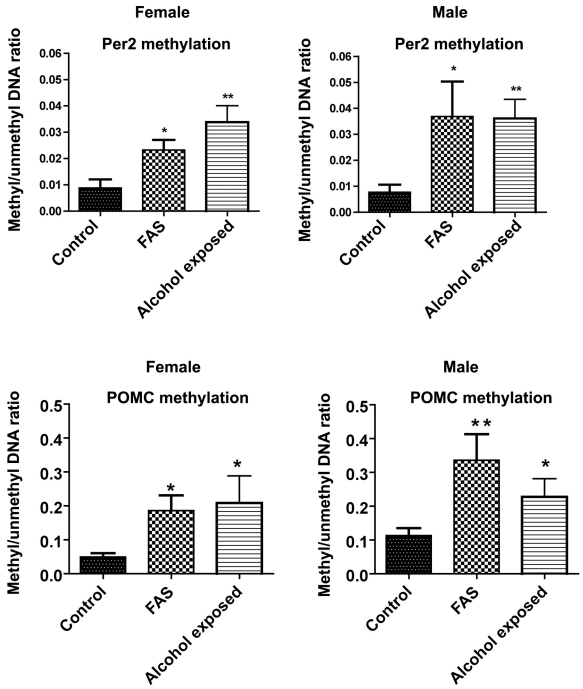
IV. Methods

We received 90 frozen salivary DNA samples (about 4 microgram each) from Dr. Tatiana Foroud laboratory at Indiana University. According to information provided by Dr. Foroud, these samples were obtained from Caucasian (either confirmed by GWAS or self report when GWAS was not available) female and male children of CIFASD cohort in the US. Of the female subjects, we received samples from 18 female and 29 male controls, 7 female and 6 male FAS subjects (as classified by the Dysmorphology core) and 8 female and 19 male prenatal alcohol exposed subjects. We performed DNA bisulphite modification and measured the CT values of the DNA. After confirming the quality of the DNA, we used them in methylation assay for Per2 gene and POMC gene.

We measured Per gene and POMC gene methylation by SYBR Green Methylation-specific (MSP) Real-Time PCR. DNA samples were undergone through the bisulphate conversion and performed with the help of EZ DNA Methylation-Direct Kit (Zymo Research, Inc). PCR primers were designed using the MethPrimer program for human (http://www.urogene.org/methprimer/index1.html). Primers were designed to be methylation-specific or unmethylation-specific with respect to the particular cytosine nucleotides in the CpG pair under analysis. The ratios of the methylation-specific to unmethylation-specific responses were quantified by ΔCt method. The Real-Time PCR was run on ABI Prism 7500 Sequence Detection System (Applied Biosystems, Inc) using PowerGreen PCR Master Mix (Applied Biosystems, Inc). 2-4 µl of bisulfate-treated DNA was utilized in each run. All runs were performed in duplicate.

V. Accomplishments and Results

The methylation analysis of Per2 and POMC genes of salivary DNA samples are completed and the data are shown in the figure below. As can be seen, Per2 and POMC gene methylation levels are significantly higher in both male and female FAS and alcohol exposed subjects, as compared to control subjects.



VI. Discussion and Significance

Because clinical sleep-wake disturbances have been observed in human neonates, children, and adolescents following prenatal exposure to alcohol, we have previously used a rat model to determine whether prenatal ethanol exposure produces long-term alterations in the circadian clock mechanism to effect the circadian regulation of endocrine functions. We found that prenatal ethanol exposure produced long-term changes in the pattern of the circadian rhythms of POMC and the clock-governing rat *Per* genes in the arcuate nucleus and the *Per* genes in the suprasciasmatic nucleus of the hypothalamus. Prenatal ethanol exposure has been shown to alter core body temperature and phase shifting ability, glucocorticoid rhythms, immune cell rhythms, and rhythmic pituitary-adrenal function. These data provide evidence for the

involvement of *Per2* in the mechanism involved in fetal alcohol altered stress control. Epigenetic modification of a gene have been shown to play a role in maintaining a long-lasting change in gene expression. Therefore, we hypothesize that alcohol's modulating effect on DNA methylation makes an epigenetic mark on *Per* genes that serves to activate the stress axis via suppression of the POMC gene leading to maladaptation of the stress system. Hence, we anticipate a significant association between *Per2* gene methylation and stress hormones (cortisol) abnormality in FASD patients. The data shown above provide exciting preliminary evidence that prenatal ethanol makes an epigenetic mark on *Per2* and POMC genes in human patients.

VII. Interrelation with Aims of the Consortium and Other Projects

We are working with other Consortium projects conducted by Drs.Christina Chamber, Tatiana Foroud and Elizabeth R. Sowell.

VIII. Plans for the Next Year

We now need to show that the methylation marks on *Per2* and POMC genes are also detectable in blood DNA samples. Furthermore, longitudinal studies need to be conducted to assess if the epigenetic modification of *Per2* and POMC genes are stable or there are changes due to treatment and/or age. Furthermore, we need to test whether *Per2* and POMC gene methylation and expression levels correlate with the stress abnormality (high cortisol and testosterone)? These studies will be crucial in determining whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

IX. Publications

Rachdaoui N, Sarkar DK. Effects of alcohol on the endocrine system. Endocrinol Metab Clin North Am. 2013 Sep;42(3):593-615. doi: 10.1016/j.ecl.2013.05.008. PubMed PMID: 24011889; PubMed Central PMCID: PMC3767933.

CIFASD 2013 Annual Progress Report: Informatics Core

I. Principal Investigator:

William K. Barnett, Ph.D.

II. Title of Project:

Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders

III. Objectives:

The objective of the Informatics Core is to support the goals of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) by enabling the collection, sharing, and analysis of consortium data. The goals of the previous CIFASD Informatics Core grant, which we executed through June, 2012 were:

1. Facilitate the creation of data dictionaries to ensure that information of different types and from different locations can be shared and integrated,
2. Develop a reliable and HIPAA-aligned central repository
3. Provide other tools to facilitate data entry, data storage, and data retrieval, and
4. Enable both hypothesis-driven and data mining methods for analyzing data.

The objectives of the CIFASD Informatics Core, beginning in July, 2012 are:

1. Continued cyberinfrastructure support. The Informatics Core will consult and collaborate with CIFASD clinical projects, cores, and developmental projects to support the existing data submission to the CIFASD Central Repository, manage those data, and make those data available for use to accomplish CIFASD goals.
2. Collection of additional data sets. The Informatics Core will securely bring new data sets into the CIFASD Central Repository for cross-study and cross-modality data analysis amongst CIFASD projects.
3. Support of affiliated projects. The Informatics Core will develop technical solutions that will allow the comparison of data between CIFASD and affiliated projects.

IV. Methods:

CIFASD is currently collecting several different types of data: Dysmorphology, Neurobehavior, 3D Facial Images, Demographics, Alcohol and Control Variables, Ultrasound, Infant and Preschool Neurobehavior, and Brain Images. For each of these categories of data, the Informatics Core worked with the rest of the consortium during 2013 to accomplish the following:

- Create and/or update data dictionaries that precisely describe data to be collected.
- Create and/or update one or more input tools that allow projects to record their data.
- Expand the ability of the central repository to store data and create and/or update methods to transfer data from the input tools to the central repository.
- Investigate new methods for retrieving data to include the ability to retrieve each type of data in turn, and new tools for data browsing directly by researchers.
- Develop reports to help identify data completeness and ensure the quality of the data in the Central Repository.

The result is a new or improved set of software tools that allows project members to store each of the types of data being collected for the consortium, upload/submit those data to a central repository, verify data integrity, and access this central repository for results obtained across the projects in the consortium.

Querying the central repository provides data sets that researchers can download and use for hypothesis-driven analysis. Additional flexibility in choosing datasets of interest and online visualization methods continue to be considered to enhance support for hypothesis-driven analysis. Supervised and unsupervised data mining algorithms, techniques, and software are also still being considered to enable researchers to find patterns and meaning in the data.

V. Accomplishments and Results:

The Informatics Core continues to provide cyberinfrastructure support by ensuring that data submissions are successful, secure, and readily available when necessary for use by the consortium members. The Informatics continues to supply and support the infrastructure that manages the Central Repository, including the servers, software such as the Oracle relational database management system, and login and security controls. All issues and change requests go to a ticket tracking mechanism, which then notifies the Informatics Core team of potential problems, therefore minimizing the possibility of requests being "lost" and not acted upon on a timely manner. This also ensures that someone in the team is always monitoring the queue and available to assist if necessary.

Data for the consortium are stored in duplicate – one copy in a robotic tape storage system in Indianapolis, IN, and a second copy in a robotic tape storage system in Bloomington, IN. This ensures that the consortium's valuable data will be kept reliably even in the event of a disaster at one of Indiana University's two computer rooms.

Several data dictionaries and tools have been created, modified, and/or improved to address the needs of consortium to add or retrieve data for cross-study and cross-modality analysis amongst CIFASD projects.

Dr, William Barnett has been asked to chair the CIFASD Data Access Committee. Other members of the committee are: William Dunty (NIH), Tatiana Foroud, Elizabeth Sowell, Sarah Mattson, and Christina Chambers. The purpose of the Committee is to recommend how CIFASD can best share data with investigators outside the consortium.

The status of the development of these data dictionaries and tools follows. An asterisk (*) represents status that has changed since the last report in January, 2013. Please notice that, if the previous status was marked finished and it is now in progress, it means that there is a change request.

Data Area	Task	Status
Infant Neurobehavior	Data Dictionary	Finished

[Bayley, Maternal Questionnaire, and Heart Rate Monitoring]	MS Access Input Tool	Finished
	Central Repository Schema	Finished
	Upload Mechanism	Finished*****
	Query Mechanism	Finished*
Preschool Neurobehavior	Data Dictionary	**Finished***
	MS Access Input Tool	**In progress***
	Central Repository Schema	**Finished***
	Upload Mechanism	**Finished***
	Query Mechanism	**Finished***
Dysmorphology	Data Dictionary	Finished*
	MS Access Input Tool	Finished*
	Central Repository Schema	Finished*
	Upload Mechanism	Finished*
	Query Mechanism	Finished*
Neurobehavior	Data Dictionary	Finished
	MS Access Input Tool	Finished
	Central Repository Schema	Finished
	Upload Mechanism	Finished
	Query Mechanism	Finished
Neurobehavior Phase II	Data Dictionary	Finished
	MS Access Input Tool	Finished
	Central Repository Schema	Finished
	Upload Mechanism	Finished
	Query Mechanism	Finished
Neurobehavior Phase III	Data Dictionary	Finished*
	MS Access Input Tool	Finished*

	Central Repository Schema	**Finished***
	Upload Mechanism	**Finished***
	Query Mechanism	**Finished***
Demographics	Data Dictionary	Finished*
	MS Access Input Tool	Finished*
	Central Repository Schema	Finished*
	Upload Mechanism	Finished*
	Query Mechanism	Finished*
3D Facial Images	Data Dictionary	Finished
	Central Repository Schema	Finished
	Upload Mechanism	Finished
Ultrasound	Data Dictionary	Finished
	MS Access Input Tool	Finished
Screener	Data Dictionary	Finished
	MS Access Input Tool	Finished
Follow-up/Outcome (2^{nd} Interview)	Data Dictionary	Finished*
	MS Access Input Tool	Finished*
Brain Imaging	Data Dictionary	Finished
	Central Repository Schema	Finished
	Upload Mechanism	Finished
Alcohol & Control (EEAC)	Update data dictionary	**Finished***
	Update MS Access Input Tool	**Finished***
	Update Central Repository Schema	**In progress***
	Update upload mechanism	**In progress***
	Update Query Mechanism	**In progress***
Eating Habit Interview	Create data dictionary	**In progress***

	Create MS access input tool	**In progress***
Cross Query	Update Cross Query Tool to minimize “Matrix effect”	**In progress**
DemGroupClass Report	Adjust to accommodate Neuro II Time Tested I and Time Tested II datasets	Finished
	Create a new report for Neuro III Time Tested I dataset	**Finished***
Tallies Completeness Reports	Create reports for phase III	**Finished***
	Create monthly automatic report submission vie email	**Finished**

The continuing development of data dictionaries is a particularly useful contribution of the Informatics Core to the consortium as a whole. Data dictionaries are critical aspects of information repositories, as they provide the necessary consistency to ensure that data from different projects can be compared. The creation of the data dictionary facilitates the process of the consortium agreeing on the meaning of the data being collected, and the definitions in the data dictionary are programmed into the tools used by the consortium in order to enforce that the data entered and submitted conform to the data dictionary. The degree of detail required to create shared data input tools has repeatedly helped us discover areas in which definitions were almost consistent rather than completely consistent. We have been very careful to leave it to CIFASD and subcommittees appointed by the CIFASD leadership to actually define the data items. This interplay has been very productive. Ultimately, these data dictionaries could become a set of standard terms for FASD research in general, and are now publicly available at: http://cifasd.org/data-sharing/.

Consortium researchers may download data dictionaries and MS Access input tools from https://cifasd.uits.iu.edu/downloads/downloads.html.

Example screen images of data entry and retrieval tools we have created are shown in Figures 1 – 4

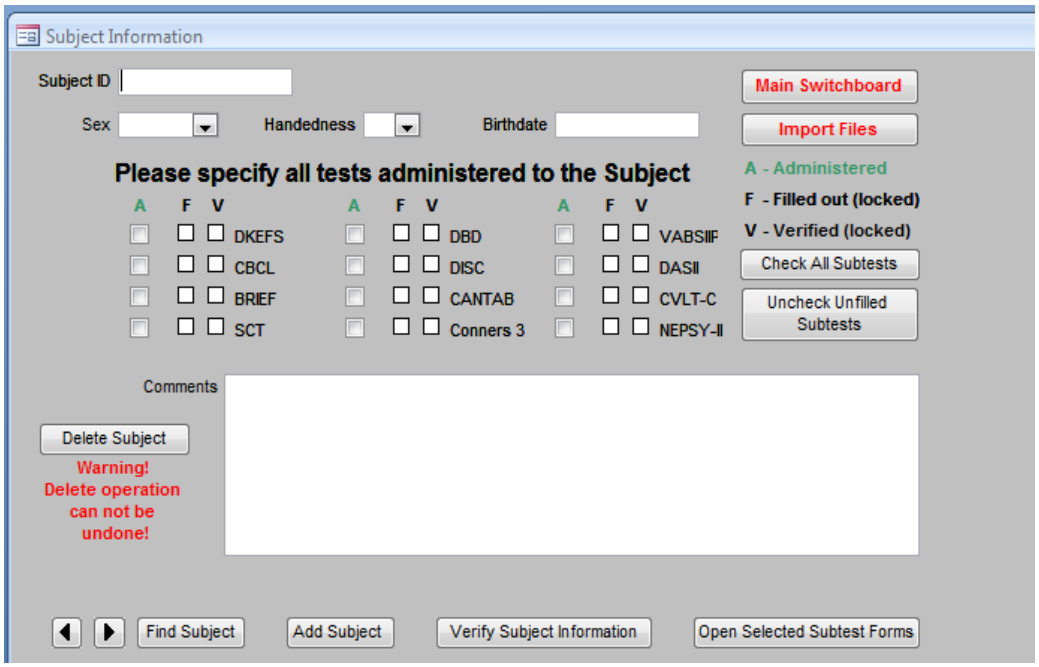


Figure 1 – New datasets implemented for Neurobehavior Phase III Access input tool

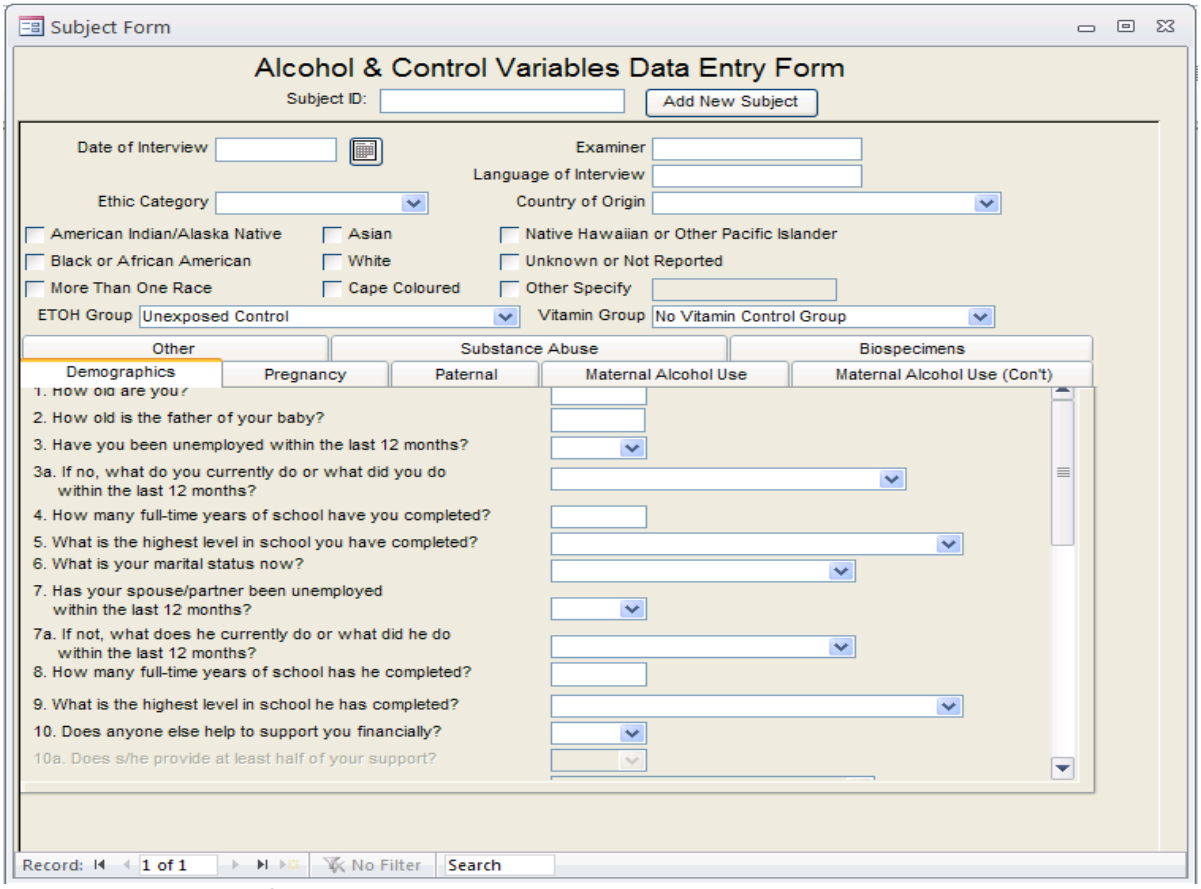


Figure 2 EEAC Data Entry Input Tool., as well as range checking that makes it impossible to enter values outside the permissible ranges identified in the data dictionary.

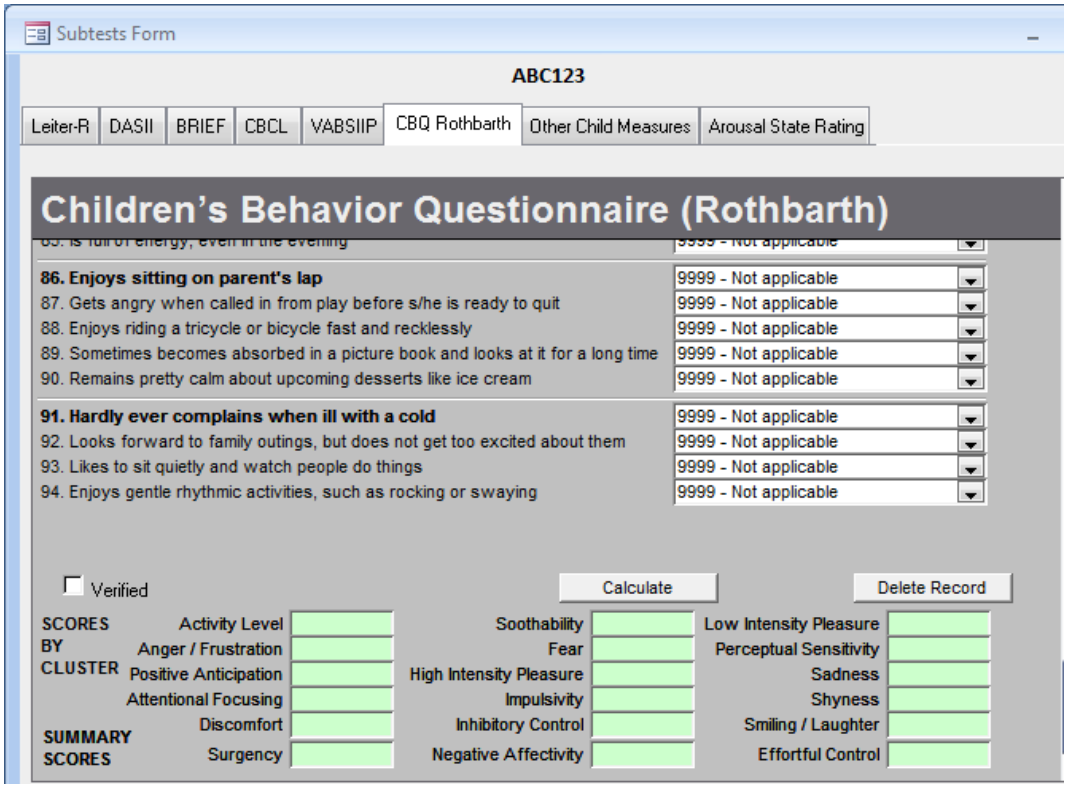


Figure 2 - Preschool Neurobehaviour Data Entry Input Tool provides validation functionality as well as range checking that makes it impossible to enter values outside the permissible ranges identified in the data dictionary.

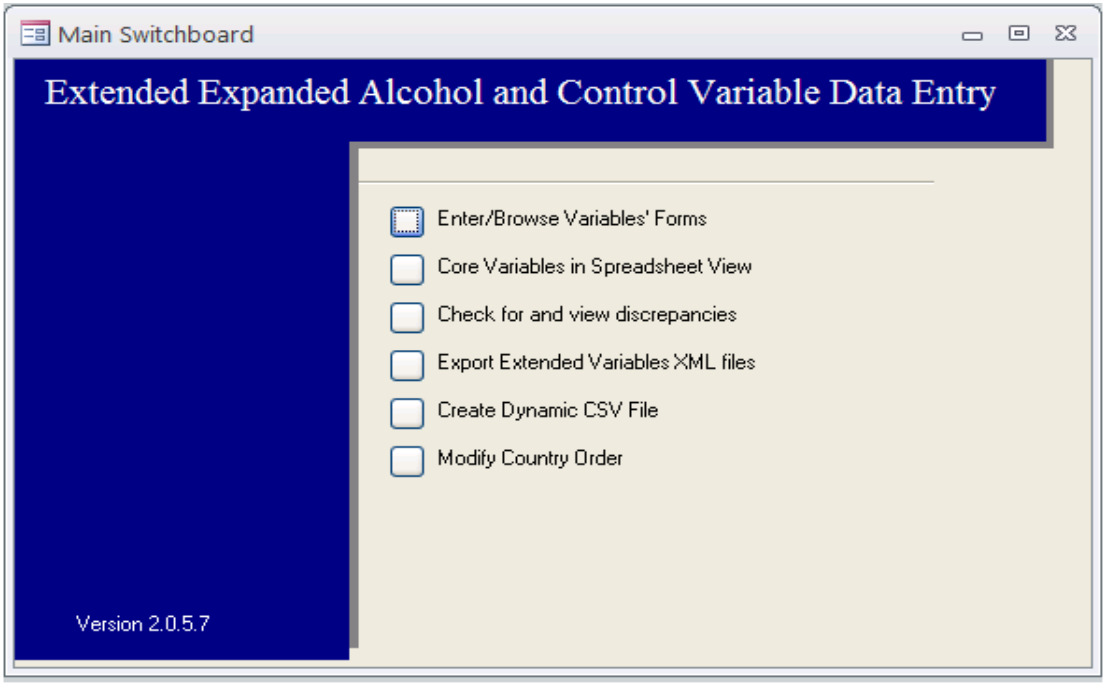


Figure 3: The EEAC Main Switchboard.

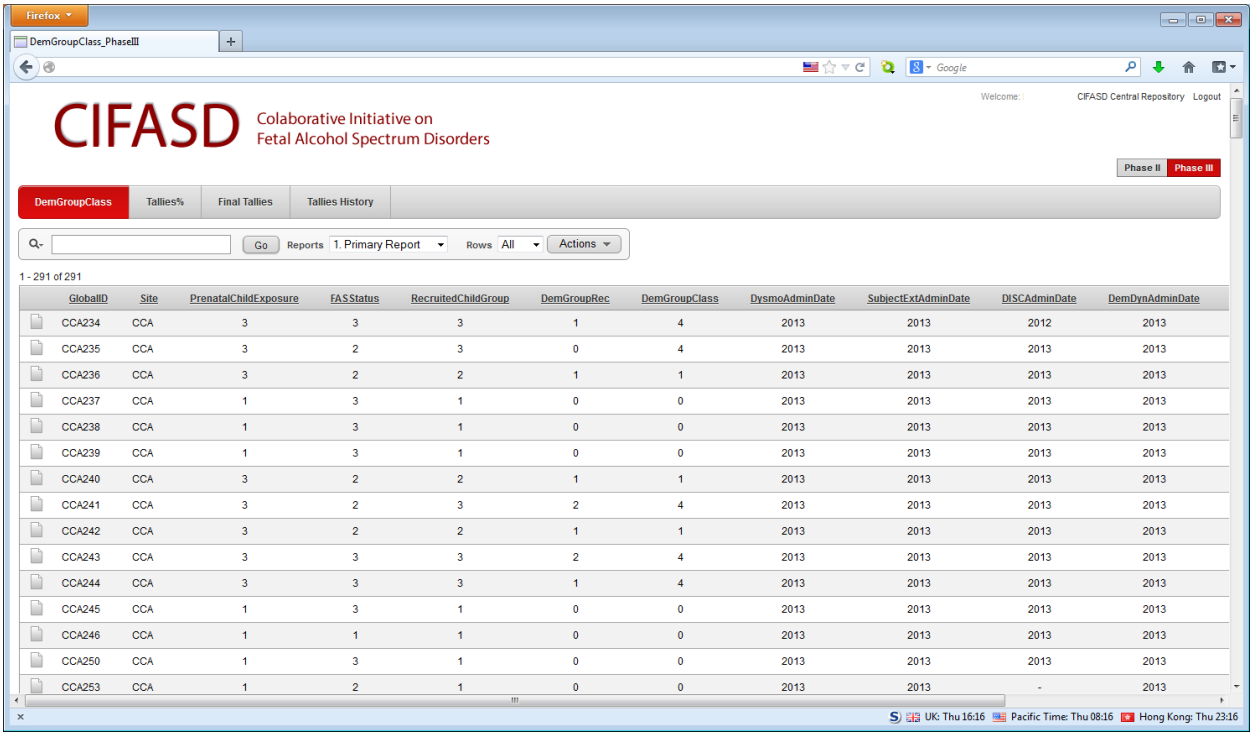


Figure 4 - The DemGroupClass Phase III report. This report is used to ensure that subjects are being consistently organized into a particular group based upon strict criteria that would yield the same classification system over all sites.

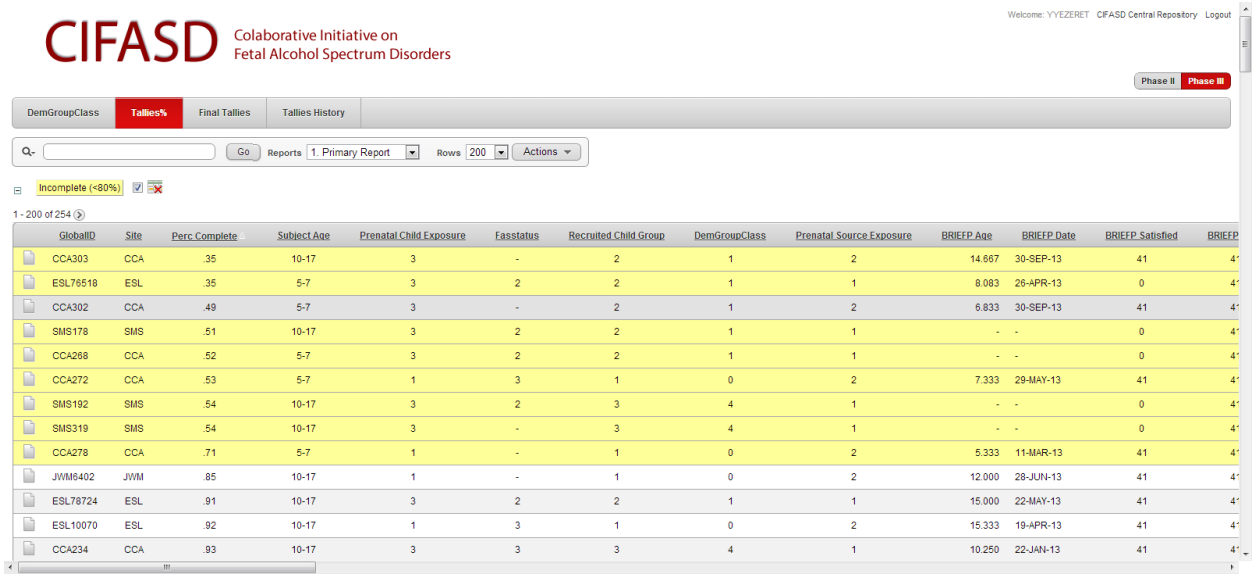


Figure 5 – The Tallies Percentage Report is a fully interactive report that depicts association between various Neuro Phase III, Demographics and Dysmorphology Exam records. The record is considered complete if total percentage of the records that satisfied the requirements of the individual subtests exceeds 85% (PercComplete column). Individual researchers can create and store reports on the page.

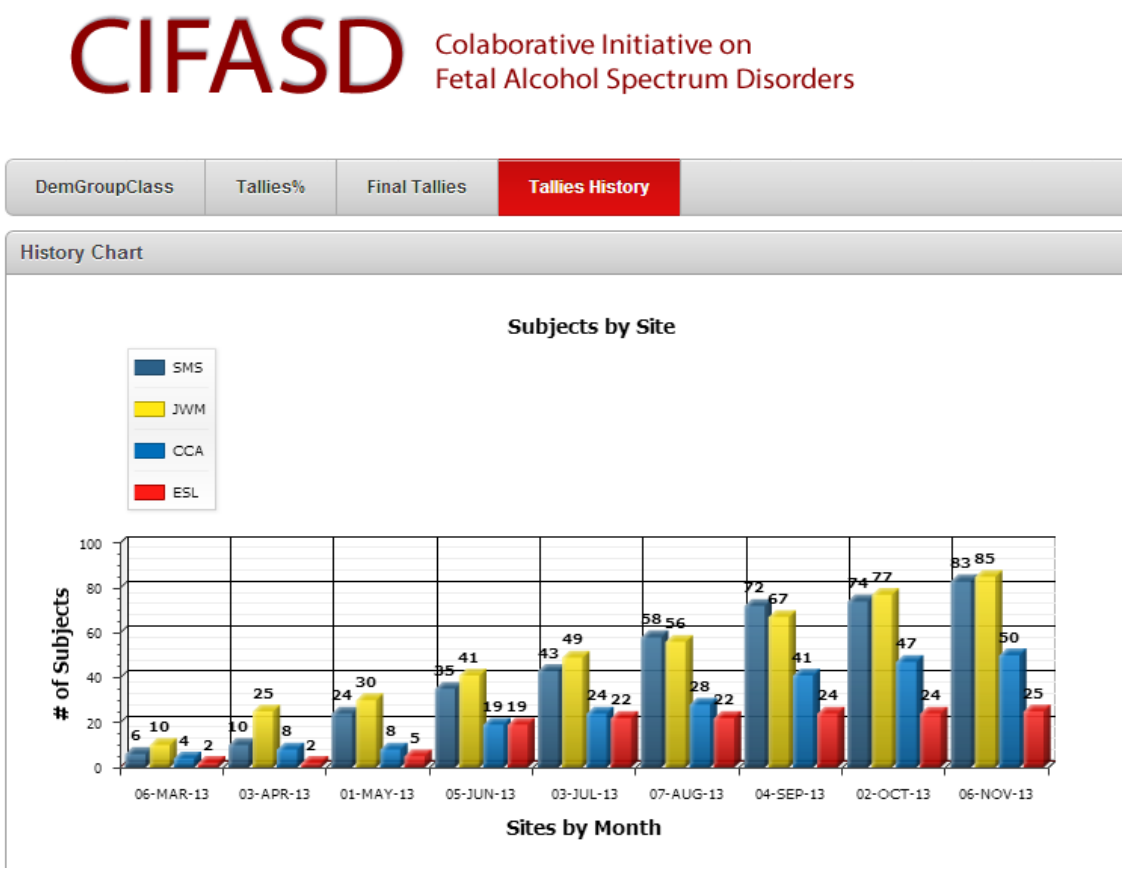


Figure 6 – The Tallies History provides a quick visual overview of the number of records submitted to Central Repository by different sites by month

Another example of new data sets that are available in the CIFASD Central Repository is Genetic Information such as DNA and GWAS, as shown in figure 7.

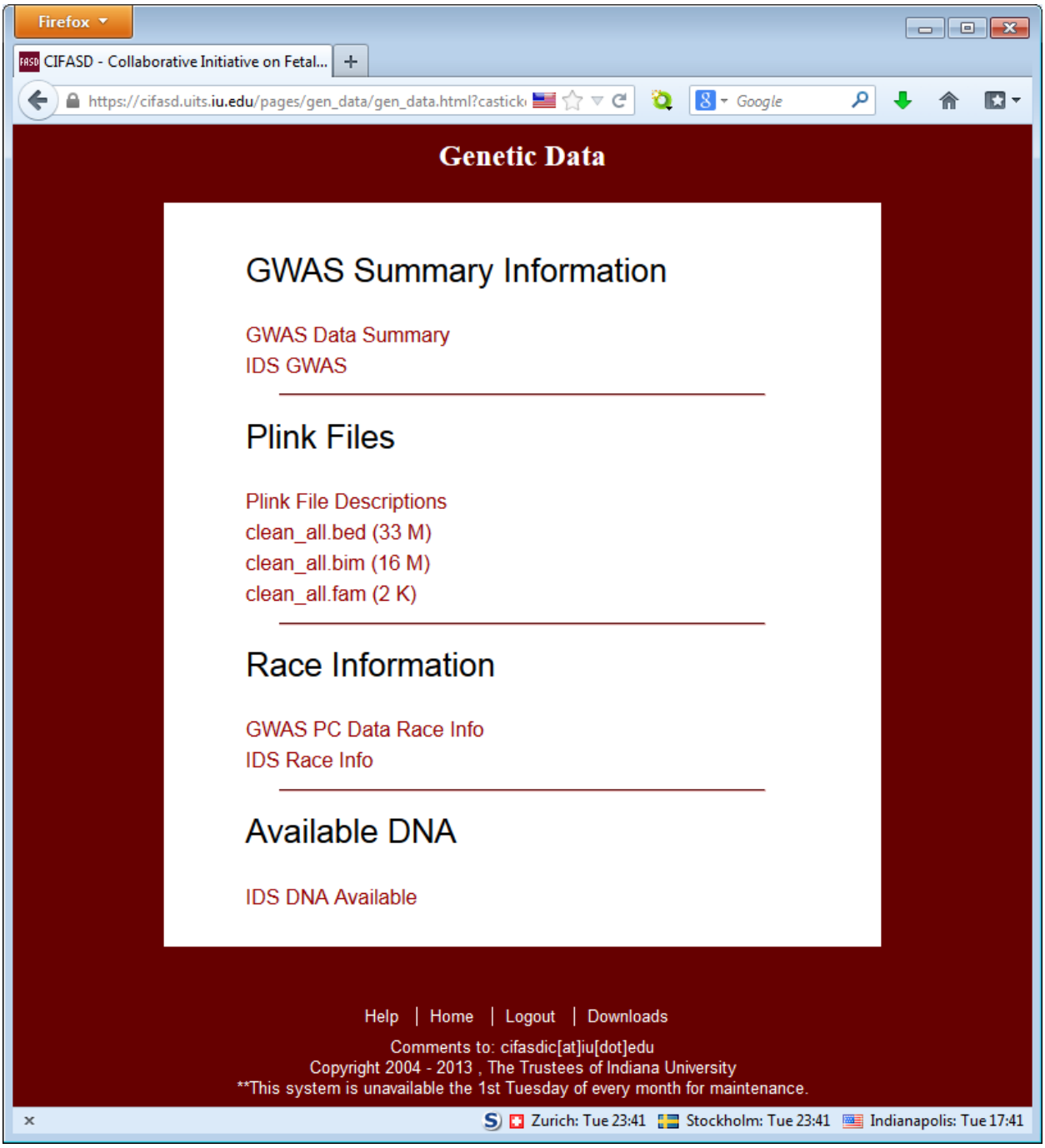


Figure 7 – Genetic data available for consortium members.

The Cross Query tool realizes the value of the integrated, consortium-wide repository by enabling researchers to select data of interest from across the range of data types and the range of participating sites and populations. The Cross Query tool provides data in multiple formats to enable researchers to use the provided data with the analysis tools of their choice.

The Cross Query tool cannot provide every possible dataset that CIFASD collaborators may be interested in, so the Informatics Core supplements the Cross Query tool with a consulting service to provide customized datasets as needed.

Although this year's focus was to create, improve, and modify the tools, the Informatics Core continues to look for technical solutions that will allow the comparison of data between CIFASD and affiliated projects. The Cross Query tool is still a priority, and the expectation is to resume work on it once other priorities to develop the tools are met.

VI. Discussion:

The initial Informatics priority for CIFASD had been the creation of tools to enable the capture and submission of data. At the start of 2013, these tools were largely created. The emphasis of effort this year was thus on continuing to collaborate with the sites to update upload capabilities based on changing consortium needs, ensure data integrity, and to provide useful sets of data for analysis.

The emphasis of the Informatics Core's work will continue to migrate from creating tools that capture and submit data to facilitating more and better methods for analysis of the data that has been collected.

VII. Interrelation with Aims of the Consortium and Other Projects:

The Informatics Core is essential infrastructure for CIFASD as a whole. The structure of a separate Informatics Core has facilitated the collaborative processes that have enabled the consortium's researchers to come to consensus on data definition and measurement issues that are essential to the broader goals of the CIFASD as a whole. We believe that this can be a model for FASD research programs in the future. The work of the Informatics Core has led to the creation of data dictionaries that will ensure that the common data collected by the consortium are usable and understandable indefinitely, the creation of a Central Repository of data from clinical research projects, and the suite of computer tools we have created that ensure the data are accessible.

VIII. Plans for the Next Year:

The Informatics Core will continue to work with the entire consortium to provide data input, management, and output services, understand the ongoing needs for software tools and their development, provide better tools for data analysis, and assist with data quality. There will be a continued need for Informatics Core staff to assist researchers with data uploads. There also will be an ongoing need for tool modification as data upload needs, methodologies, or contributing sites change. As projects create new data types, such as genomic data, and affiliate projects create data integration requirements, the Informatics Core stands ready to assist in those endeavors.

The focus of the consortium continues to shift from the emphasis on collecting to analyzing and comparing data among affiliated projects. The Informatics Core will continue to be a facilitator for CIFASD clinical projects, support existing data submissions, and develop technical solutions for new datasets and for cross-study and/or cross-modality data analysis. In addition to creating and/or modifying existing input and upload tools, we will also remain focused on improving query and reporting capabilities for both hypothesis-driven analysis and quality assurance. The CrossQuery system supports integrated queries across multiple data sets.

We will continue supporting an option of retrieving any individual (or all) fields from a selected dataset. We are also working on eliminating what is called the “Matrix Effect”, the phenomenon in which the CrossQuery attempts to create all possible interactions between results when, in reality, we only want one line of data for each expected result (think Punnet square). By addressing this issue, we will improve confidence in, and enable greater engagement with, CIFASD researchers, thus promoting data use. We will also explore other possible formats that can be used to export data (such as SPSS).

The Informatics Core will also continue to provide ongoing support for data quality improvements. We will focus on improving the data browsing tools so that the researchers can directly examine the data sets they have uploaded to the Central Repository. We will also allocate time, as needed, to directly support the improvement of data quality in the Central Repository by, for example, creating specific new reports or improving existing ones such as DemGroupClass and the Tallies Completeness.

IX. Publications:

n/a

X. Posters and Presentations:

n/a

XI. Supplements, Training, and Community

n/a

I. Principal Investigator: Kenneth Lyons Jones
II. Title of Project: Dysmorphology Core
III. Objectives/Specific Aims/Goals

Specific Aim #1: To assure consistency as well as accuracy in recognition of Fetal Alxohol Specturn Disorders (FASDs) at all CIRFASD project sites throughout the world.

For this specific aim the Dysmorphology core:

i. Use the previously established CIFASD physical examination protocol and classification system to perform and/or validate physical examinations of all infants and children who were participants in the CIRASD renewal project who have not previously been examined by the Core examination team.
ii. Use the previously established CIFASD examination training protocol to provide on-going training and re-training of local pediatricians/neonatologists/geneticists who are providing preliminary examinations at some CIFASD sites.

Specific Aim #2: To develop a training DVD that could be used to teach physicians and other health care professionals with little or no experience in diagnosis of FASD to correctly identify the characteristic structural features of FAS through a physical examination and to successfully diagnose or rule out this disorder on that basis.

For this specific aim the Dysmorphology core:

i. Will develop a training DVD through the help of the Office of Continuing Medical Education at the University of California San Diego.

ii. Will compare the effectiveness of that training DVD with a hands-on live training program done by a member of the Dysmorphology core. That will be accomplished by comparing pediatricians who have completed training with the DVD to pediatricians who have been trained by a member of the dysmorphology core. They will be compared based on their ability to correctly identify features of FASD as well as their ability to make a diagnosis of FASD.

Specific Aim #3: To develop a methodology whereby long-distance consultation can be provided to physicians and other health care providers in outlying areas throughout the world.

For this specific aim the Dysmorphology core:

i. Will develop, with the help of the Telemedicine Communications Center at UCSD, a wireless, interactive, audiovisual tele-consultation system that would provide the opportunity for a practitioner in one remote locale to perform a comprehensive examination of a child being evaluated for FASD that could be simultaneously viewed, commented upon and corrected by an expert dysmorphologist at a central location.

ii. This system will initially be developed and field-tested in San Diego, but will then be available for trials in other more distant areas.

Specific Aim #4: To document the prevalence of major malformations in children prenatally exposed to alcohol, and in so doing, delineate the extent of Alcohol Related Birth Defects (ARBD).

i. For this specific aim the Dysmorphology core will determine the prevalence of ARBD in subjects ascertained through the prospective cohort study in Ukraine. 200 preschool age children born to moderate to heavy drinking mothers and 207 preschool children born to low/unexposed mothers will be available for physical examination and ultrasound evaluation as appropriate to identify/confirm cardiac defects, oral clefts, renal anomalies, and/or more subtle manifestations of other defects.

IV. Methods:

We have performed a standard comprehensive physical examination of all children at all consortium sites. These children were examined blind to alcohol exposure in all cases. Based exclusively on the standard physical examination, each child was categorized as FAS, No FAS or Deferred. The latter category will be used to better understand the full spectrum of defects resulting from prenatal alcohol exposure.

Although serious problems in neurodevelopment are the most devastating consequence of prenatal alcohol exposure, diagnosis of Fetal Alcohol Spectrum Disorders is dependent on a careful physical examination performed by a dysmorphologist with expertise in this diagnosis. Assurance that all children are appropriately evaluated is imperative relative to the integrity of conclusions that can be drawn regarding the overall aims of the CIFASD Consortium. The current project is focused on training additional physicians with the goal to take the burden off the few dysmorphologists with expertise in making this diagnosis.

Initial efforts have included the development of two training videos. The first is one to teach physicians and other healthcare professionals an approach to correctly identify the characteristic structural features of FAS through a physical examination providing examples of standardized measurements and photographs to successfully diagnose or rule out this disorder on that basis. The second is one to teach physicians how to film the physical exam and what to include when videotaping the clinic session for consultative review with the expert dysmorphologist. The accompanying handbook documents procedures for consents, physical examinations and preparation of videos for transmission.

We are now proceeding with the development of a tele-communication system where-by physicians in remote areas with little or no expertise in diagnosis of FASD can perform a physical examination of a child being evaluated with FASD that could be simultaneously viewed, commented upon, and corrected by an expert dysmorphologist at a central location. This will provide an innovative approach to the diagnosis of FASD in underserved areas throughout the world where physicians with expertise in diagnosis of this order are unavailable.

To complete the project specific aim of building a clinical system configuration that supports the practice of telemedicine in areas throughout the world, we need to meet the various requirements for network speed, encryption, and security to ensure the integrity of the images and confidentiality of participant information. The system and network hardware, as well as the communication and clinical collaboration software must comply with established industry protocols and certifications.

V. Accomplishments and Results:

Specific Aim 1: Over the last 6 months we have provided accurate recognition of FASD at two sites in Ukraine, at San Diego State University, the University of Minnesota, and at Emory University in Atlanta, Georgia. All of these children have been evaluated using a direct hands on physical examination. During trips to Ukraine we have expended a considerable amount of time interacting with pediatricians and geneticists in both sites, Rivne and Khmelnitsky. We have been very pleased to note that our examination of children at both sites has been completely consistent with the examinations performed at the time of birth by the pediatrician and geneticist that we have trained. We have also been involved in re-training pediatricians at both sites.

Specific Aim 2: With respect to the development of a training DVD that can be used to teach physicians and other health care professionals to correctly identify features of FAS, we have developed a 20 minute DVD of a neonate and a 20 minute DVD of a 6 year old child that demonstrates a careful Dysmorphology examination that focuses on minor malformations in structural development. Having shown that this can be developed and that it is effective in training Pediatric residents, we are developing a shorter version which focuses entirely on minor malformations that are diagnostic of FAS. We have now developed a DVD of a 6 month old African-American child with FAS and are in the process of developing additional DVDs of children with FAS at different ages and different ethnic groups.

Specific Aim 3: Work has begun to develop and implement the tele-communication system in two consultation functional phases with the building, extension of the physical clinic space and network infrastructure occurring concurrently to support the consultation phases.

Phase I: Pilot Consultations

This phase gives remote physicians and healthcare providers the capability to film the physical exam, complete the required accompanying documents, and securely transmit them via the internet to Dr. Jones for review and schedule a consultation. For this consultation, the remote clinicians will be looking at the participant documents and video at their site and Dr. Jones will be looking at the same information on the secure repository at his site.

To ensure quality control of capturing and conducting consultations, protocols and procedures will be emphasized during training. A comprehensive DVD will be included as part of the official training materials. Participant consent forms will be required for video/photo capturing and FASD exams. This video consent will be given only to consented participants by local project staff. Exams will be recorded in a consistent process by following specifications detailed in the telemedicine handbook. The handbook will also document best practices for converting and uploading videos, including compression schemes, encryption and format recommendations.

After the proper consent forms have been obtained and the exam has been documented to video, the resulting data (or “participant packet”) will be transferred via internet to a secured remote storage repository, and an email notification will be sent to Dr. Kenneth Jones, the dysmorphology specialist in San Diego to schedule a consultation.

The storage repository will be equipped with SharePoint data management software, which will enable secure data sharing between study locations. Network security will utilize AES encryption compliant to U.S. HIPAA (The Health Insurance Portability and Accountability Act of 1996) standards to protect the confidentiality of participant health information. The videos will be shared only with Dr. Kenneth Jones. As part of security protocol, videos will be destroyed after a completed consultation, and participant consent forms will be de-identified and kept for data analyses only.

The basic network and system infrastructure to support wired connectivity for the Phase I: Pilot Consultations is in place at Khmelnitsky Prenatal Clinic. At this time there is a clinician identified and a dedicated camera for project use at the Khmelnitsky Prenatal Clinic. Some network upgrade is proposed to improve the quality of the transmissions and joint consultative session. The network and system configurations for the clinics are detailed here with recommendations for upgrades that would improve Phase I performance and prepare for Phase II.

Khmelnitsky Prenatal Clinic

Primary contact: Fedun Igor Viyacheslavovich (fedun_igor@inbox.ru)

IT support is provided by the ISP HappyNet (happynet.com.ua) for desktop computers on the first and fourth floors only. Initial speed tests on wireless network (8.14/1.57 Mbps download/upload) indicate insufficient bandwidth. Current LAN (wired network) bandwidth for first and fourth floor is 10 Mbps. This should be upgraded to a minimum of 30Mbps ($10 USD/month) or 50Mbps ($15/month). A dedicated IP address for the telemedicine station will cost $36 USD/year.

Remaining floors of the clinic have minimal internet access and are supported by a different Internet Service Provider (ISP), and will likely not be able to provide the network access needed for the telemedicine station. That is okay for this project as the existing exam room will support the required technology and clinical examination space.

Support for Cisco equipment is provided by MUK in Kiev, Ukraine. They work directly with Igor and will be the point of contact for cost, certification and customs questions regarding the telemedicine station(s). We need to provide them with an equipment list to confirm support for our preferred hardware.

Ukraine, Kiev, 03151, 16/2, Donetska str.
tel: (044) 492-29-29, 594-98-98 (multichannel)
muk.ua/contact_us/
office@muk.com.ua
cisco@muk.ua

The basic network and system infrastructure to support wired connectivity for Phase I: Pilot Consultations is not currently in place at the Rivne Diagnostic Center. Also there is also no clinician identified to run project and a dedicated camera that can be used for telemedicine clinical consultation. The necessary network upgrades to support Phase I connectivity are summarized here. It is not known how difficult it will be to proceed with obtaining the services required to support telemedicine requirements and the estimated recurring costs exceed project budget.

Rivne Diagnostic Center

Primary contact: Nick Sirochuck (vogd@rambler.ru, vogdjara@gmail.com)

IT support is provided internally by Nick as part of the IT department. Support includes reconfiguration of firewalls which might block telemedicine station connectivity. Internet service is provided by Volia at 50 Mbps and can be upgraded if needed, but there are only 100

connections for the entire building (all staff and admins have connections). Temporary connections are available in exam rooms but must be requested by the head doctor and approved by the IT department. Wireless internet is available only to IT department members.

Physicians are currently using TrueConf for video conferencing. TrueConf is cloud-based software provided by the Ministry of Health. An account is required and costs $2,000 USD/month or $10,000 USD/year.

Phase II: Real-time Consultations

This phase will give physicians and healthcare providers the capability to perform the clinical examination and consultation with Dr. Jones during a live session at the remote site. Dr. Jones will be viewing the participant and consulting with clinicians to make the FAS diagnosis or successfully rule this disorder out. Real-time consultations will give clinicians in the remote sites the benefit of interactive consultation with the participant present. The hope is that this will be an effective clinician training tool and a more effective way to determine the next steps for the participants and their families.

Real-time consultations will follow protocols and procedures similar to pilot consultations. Appointments will be scheduled via email. Consent forms will be required prior to conducting and recording examinations and sent with compressed media files to the secured remote storage repository as part of an encrypted participant packet. As with the pilot consultations, videos will be shared only with Dr. Kenneth Jones (and be deleted upon a completed consultation), and participant consent forms will be de-identified and kept for data analysis only.

This project phase proposes to utilize a telemedicine cart that is equipped with the computer system hardware, software and camera to support the real-time consultation. The recommended products are produced by Global Med.

LiteExamTM Mobile Telemedicine Station

Information taken from the Global Med web site at bit.ly/18ZjhFY

- Clean and compact, the LiteExamTM Mobile Telemedicine Station is an excellent all-in-one system to get you started.
- LiteExam houses a video codec and/or PC neatly within a cabinet, topped by a single screen monitor. The LiteExam facilitates mobility within small spaces.
- LiteExam is engineered with ease-of-use, interoperability and plug-n-play capabilities. Each system can be modified to address different requirements depending on the specific application.

TotalExamTM HD Examination Camera

Information taken from the Global Med web site at bit.ly/16XQtQX

- Weighing in at just four ounces, the TotalExamTM HD is the first true HD video examination camera for use in telemedicine. The sleek, modern look and small size is designed with the comfort of the user in mind. It offers ground-breaking video technologies until now only available to professional studios and HD television stations.

- It is faster and easier to acquire the best freeze frame images because the camera's count back frame analysis automatically selects the clearest view among 17 frames.
- Still images obtained from the camera's superior resolution are six-times the clarity of standard definition cameras, making features crystal clear upon enlargement.
- All of the camera's function buttons are conveniently located on top, making image acquisition and adjustment a simple one-handed operation.
- The integrated auto-focus takes the guesswork out of capturing the clearest images and, when desired, can be turned off.
- Go green with TotalExam HD. The LED light carousel uses as little as 8 watts of power compared to fluorescent lighting in other cameras that consume 40 watts.

We are working with vendor on pricing and current availability within the Ukraine. Current industry standard hardware, software and network products are made by companies like GlobalMed, Nexus and Cisco. Certifications may be required for equipment import, electrical specifications, and training operations. Physicians and IT support teams will need specialized training on hardware, software and network tools.

In order to implement Phase II the clinic sites will have to upgrade the network capacity with increased bandwidth, additional internet addresses and dedicated lines. These are recurring costs needed to support the telemedicine cart functionality and provide real-time consultative sessions. Additional network equipment will need to be purchased to support a wired or wireless configuration for this phase. As noted earlier, the costs and work required to upgrade the Khmelnitsky Prenatal Clinic are within the project budget and are upgrades to existing services. The services and equipment to provide network services to the Rivne Diagnostic Center exceed project scope and budget.

In addition to network upgrades and electrical wiring improvements, the Rivne Diagnostic Center would need to undertake physical remodeling in the clinic exam room(s) to accommodate the proposed telemedicine cart. There would also need to be a decision made on how many rooms would accommodate this functionality. Facility preparation costs would need to be covered by each clinic in readiness to extend the telemedicine capabilities.

For Phase II one basic telemedicine cart will be purchased with camera functionality. The telemedicine cart is the basic building block in integrating other monitoring functionality in system integrated diagnosis. This meets the project objective and is within project budget. Given the certification, security and network restrictions this is not a current option for implementation within the Ukraine. Acquisition and implementation of this functionality for the pilot will have to take place at a clinic site within the U.S. to meet time-constrained project objectives.

The vendors do provide optional clinical monitoring equipment components that can be interfaced to their proprietary system software for integrated consultation, ie. Ultrasound, EKG. The acquisition and implementation of this functionality is beyond the scope of the current project.

The existing clinic software and associated database used to collect participant information will be used to store videos, still images and associated documentation. An integrated software system and common repository that electronically interfaces with the telemedicine equipment and manages the videos, still images and associated participant clinical information is available from vendors and can be considered for a future project.

VI. Discussion and Significance:

The Dysmorphology Core has helped to facilitate the goals of the Consortium and some of the individual projects primarily through traveling to Consortium sites and performing standardized physical examinations on children ascertained at the individual sites. Overall the Dysmorphology Core has performed 3262 standardized physical examination on 2253 unique subjects at consortium sites throughout the world. 265 of those children have been examined since June 2013. That has included 55 children in Atlanta, 15 children in Los Angeles, 143 children in Minnesota, 37 children in San Diego, 12 children in Khmelnytsky, and 3 children in Rivne. We have given a number of talks and training sessions in Ukraine. During the last 6 months we have developed a training DVD in San Diego, CA and we have spent considerable time interacting with telemedicine technicians in San Diego and Ukraine.

VI. Interrelation with Aims of the Consortium and Other Projects:

The Dysmorphology Core has interrelated with a number of other projects in the Consortium including the 3D Facial Imaging Project, the Spectrum of and Nutritional Risk Factors for FASD in Ukraine Project, the Multisite Neurobehavioral Assessment of FASD Project, the Prenatal Ultrasound studies in Ukraine and the Neuroimaging Core.

VIII. Plans for the Next Year

1. We will continue to see children ascertained at all CIFASD sites throughout the world.

2. We will begin to access the effectiveness of the training DVD and will create additional DVDs on children of different ages and of different ethnic group.

3. We will continue work on the development of the telemedicine program. I have begun discussions aimed at developing the ability to use telemedicine to evaluate children with possible FAS at Emory University in real time. There are a number of advantages to getting this system started at Emory as things are getting set up in Ukraine. These include the three hour time difference as opposed to the 10 hour time difference between San Diego and Ukraine and the ability to evaluate the same children by a direct physical examination at Emory and by long distance physical examination using telemedicine between Atlanta and San Diego. Finally, we will initiate investigation of the incidence of Alcohol Related Birth Defects in children prenatally exposed to alcohol.

IX. Publications

Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN. “Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD.” Neuropsychology. 2013 Nov;27(6):713-24. Doi: 10.1037/a0033994. Epub 2013 Sep 16, PMID: 24040921.

X. Poster Abstract References and Presentations

Jones, KL, Chan, PR, Yevtushok, L, Zymak-Zakutnya, N, Wertelecki, W, Keen, CL, Chambers, CD “Mechanism Involved in Variable Craniofacial Phenotypes Following Prenatal Alcohol Exposure: From Mouse to Human.” Presented at the 2013 David W. Smith Workshop on Malformations and Morphogenesis, Quebec, Canada, August 2013.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Non-compliant	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN. Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921.

I. Principal Investigator: Christina Chambers

II. Title of Project: Early Identification of Affected Children and Risk Factors for FASD in Ukraine

III. Objectives/Specific Aims/Goals

Aim 1 Determine if beneficial effects of a prenatal MVM supplementation intervention in alcohol-exposed children persist at preschool age.
Aim 2 Determine if prenatal alcohol exposure in the context of the prenatal nutritional environment affects child nutritional status and determine the extent to which nutritional status in children prenatally exposed to alcohol affects growth and performance.
Aim 3 Determine if a miRNA biomarker for alcohol exposure previously identified in a shepp model translates to the human as a marker of recent or distant exposure to various levels of alcohol.
Aim 4 In collaboration with CIFASD investigators, explore selected genetic and epigenetic risk factors for FASD, the effect of nutrtion on 3D facila images, and telemedicine approaches to early diagnosis.

The major goals have not changed since the initial competing award or previous report.

IV. Methods

Aim 1 Recall children previously tested with the Bayley Scales of Infant Development in infancy, and retest at approximately 3.5 – 5 years of age with a preschool testing battery.
Aim 2 Recall children previously enrolled in the study at 2 years and again at 3.5 – 5 years of age and collect information on current dietary intake and growth. Enroll new pregnant women not randomized to the vitamin intervention and collect within pregnancy and child measures as needed to incorporate into the sample for Aims 1 and 2. Collect blood, urine and saliva/skin cells from children for analysis.
Aim 3 Select maternal plasma samples from previously archived material and in Year 2 analyze first set of samples for evidence of a miRNA pattern. Select child samples paired to those mothers' sample in Years 2 and 3 as they become available for analysis of miRNA.
Aim 4 Select maternal/child samples for DNA extraction, collect new maternal and child samples for DNA extraction and analyze data; collect 3D images at one site.

V. Accomplishments and Results

We have cumulatively recruited 62 new exposed pregnant women and 50 new unexposed pregnant women across both sites for a total of 112 women and blood samples as well as interview data have been collected. This exceeds our goal through the end of year 2, and exceeds our goal for the entire five-year project period for alcohol-exposed women. This expedited recruitment allows us to enrich the pool of pregnant women and children for the study. By recruitment much earlier in the project period than we originally planned, we expect to have >120 additional 2 year olds available for evaluation by project year 5, most of whom were not originally expected to be old enough to participate.

We have cumulatively evaluated 25 children at the 2-year or 4-year visits. As of November, 2013, the Khmelnytsky site has fewer than 10 children who are already old enough for the 4 year visit, so they have worked on establishing competency in their neurobehavioral tester with practice. The Rivne site has more children eligible for the 4 year old visit, but to ensure tester's

competency, DVDs of the testing battery administration are being recorded and scoring materials scanned for review by Claire Coles and Julie Kable. Weekly/monthly feedback to the testers is being given via skype video conferences. In addition to the three face-to-face trainings for the testers that have already taken place in Ukraine, this additional intensive training has been deemed necessary to obtain confidence that the testing battery can be completed in a timely fashion as well as scored accurately. The heart rate monitoring equipment is in place, testers are functioning adequately, and these data are being collected at both sites for infants and 3.5-5 year olds.

Maternal interviews, 24 hour dietary recall forms, and growth measurement forms have been translated and are in use. The CIFASD Repository database has been finalized to accept these new measures and the preschool battery neurobehavioral input tool has been tested with practice data in the last month.

Protocols and training for the various biological sample collections, preparation and shipping are in place, translated to Ukrainian and posted to the study intranet site; appropriate supplies have been purchased and delivered to the sites for blood, urine, and buccal swab/saliva collection. The partners in Ukraine are still working on best techniques for getting compliance from women for the return visit, and for obtaining enough blood from a capillary stick if that is all that is possible, especially in the 2 year olds. We are returning a feedback letter on the nutrition results from within pregnancy to each of the mothers, and plan that the neurobehavioral testing in 4 year olds be scored and results returned to parents within the week after testing as an incentive to return.

The archived samples of plasma and white cells from approximately 500 participants have been evaluated for relevance to the study objectives and distributed by UC Davis. 500 white cell samples were delivered to Kelly Frazer's lab at UCSD in the last two months, and the strategy for analyzing these most cost efficiently is now being reviewed. Plasma samples for the miRNA study by Miranda have been selected from the pool, and these have been sent to Dr. Miranda's lab at Texas A&M as of December, 2013.

Approximately 60 3-D facial images have been captured in Khmelnytsky where the camera now resides. Following discussion on disposition of images with Drs Foroud and Hammond, an agreement has been reached with the Ukrainian partners. The images will now be stored on mobile media and shipped to Indiana within the next month.

Telemedicine protocols and step by step plans for implementation were discussed at the site visits to Ukraine in April, 2013 and again in September, 2013. See Dysmorphology Core progress report.

We continue to move toward publications. We currently have one paper in press summarizing the results of the screening of >10,000 women and predictors of risky alcohol use, and one paper on Vitamin D status that has been returned for response to reviewers. Six additional publications are in various stages of final analysis of data or completion of discussion sections.

VI. Discussion and Significance

Implementation of the much more complex protocols in this renewal of the Ukraine cohort study has been time consuming under difficult administrative and political circumstances. Findings of the ongoing analysis of the maternal data and infant outcomes continue to be highly promising; but complex in interpretation, especially as they relate to choline supplementation.

The screening paper that is in press was requested for use by USAID as the premise for designing a prevention/intervention program in Ukraine as this is the only current and the most comprehensive data ever published on the prevalence of alcohol exposure in pregnancy in that country. The findings suggest that the prevalence of risky alcohol consumption in pregnancy is high, and help inform the utility of brief screening tools in that setting for identifying at risk women. As part of this project, the acting Director of NIAAA was invited and made a site visit to one of the study performance sites and to the Lviv National Medical University in September of 2013. The impact of this project and Dr. Warren's visit has stimulated more aggressive public health efforts towards education and prevention of FASD, and continues to be a positive influence on local initiatives.

VII. Interrelation with Aims of the Consortium and Other Projects

This project compliments the neurobehavioral project of Dr. Mattson, directly supports the 3-D imaging project of Drs. Foroud and Hammond, and directly supports the Telemedicine effort of Dr. Jones. We have consulted with Drs. Foroud and Eberhardt regarding potential approaches to evaluating maternal genetic/epigenetic markers, and we rely on the informatics team to create and modify data entry tools, evaluate data uploads and quality of submissions. I am also a member of the Data Sharing committee.

VIII. Plans for the Next Year

In the next twelve months of the reporting period, we plan to accomplish the following:

1. Accelerate recruitment of children for the 2 and 4 year old visits especially in the next six months in order to meet our projected goal of 90 at each age.
2. Perform initial analysis on quality of biological samples from children.
3. Generate preliminary results from Aim 3 and the genetic/epigenetic portion of Aim 4.
4. Provide an additional ~90 3D images to Drs. Foroud and Hammond by moving the camera to the site where most number of children are being recalled.
5. Continue with planned and in progress analyses of existing data and publication.
6. Host a post-doctoral scholar from Dr. Kathy Sulik's lab who will accompany us to Ukraine in March/April 2014 to spend a week at the clinical sites learning about the study processes.

IX. Publications

1. Chambers CD, Yevtushok L, Zymak-Zakutnya N, Korzhyski Y, Ostapchuk L, Akhmedzhanova D, Chan P, Xu R, Wertelecki W. Prevalence and predictors of maternal alcohol consumption in 2 regions in Ukraine. 2013. *Alcoholism Clinical and Experimental Research*, In press. Early View, Article first published online 3 DEC 2013: http://onlinelibrary.wiley.com/doi/10.1111/acer.12318/pdf

X. Poster Abstract References and Presentations

1. Society of Maternal Fetal Medicine Annual Meeting – February, 2013, San Francisco, California – invited speaker: "Fetal Alcohol Spectrum Disorders: How Broad is the Spectrum and How Much Alcohol is Too Much?".
2. UCSD Pediatrics 4th Annual Research Symposium – February, 2013, San Diego, California – invited speaker: "Maternal Nutritional Status and the Prenatal Growth Trajectory Set in Motion".
3. OTIS Annual Meeting – June, 2013, Tucson AZ – invited platform presentation: "Multivitamin use and FASD prevention".
4. RSA Annual Meeting – June, 2013, Orlando FL – symposium presentation: "Vitamin D

Status in Pregnancy Among Women Who Consume Alcohol in Ukraine".

5. South Korea Catholic University Psychiatry and Substance Abuse Group – July, 2013, Seoul, South Korea - invited presentation: "The Epidemiology of FASD".
6. DW Smith Workshop – August, 2013, Quebec, Canada – platform presentation: "Mechanisms Involved in Variable Craniofacial Phenotypes Following Prenatal Alcohol Exposure: From Mouse to Human".
7. Lviv National Medical Center Symposium on FASD – September, 2013, Lviv, Ukraine – invited presentation: "Fetal Alcohol Spectrum Disorders in Ukraine: From Research to Prevention".
8. ESBRA meeting – September, 2013, Warsaw, Poland – symposium presentation: "Maternal alcohol consumption during pregnancy, nutritional status and impact on infant outcomes".
9. San Diego County We Can't Wait 4th Annual Early Childhood Mental Health Conference – September, 2013, San Diego, California – keynote speaker: "Maternal Exposures in Pregnancy: Impact on Early Childhood Mental Health and Development".
10. International Perinatal Mental Health Meeting Marce Society at Northwestern University – November, 2013, Chicago, Illinois – invited speaker: "Nutritional Supplements and Modification of Alcohol-Induced Developmental Deficits".

XI. Supplements, Training, and Community

There are no revisions or supplements associated with this project during this reporting period.

Several opportunities for training and development have arisen during this reporting period. These include the following guest lectures in courses for medical students, graduate and undergraduate students at UCSD:

1. UCSD-Child Health and Development Organized Research Unit iDEV Seminar – May, 2013
2. UCSD-Preventive Medicine Residency Program – September, 2013
3. UCSD-USP144 – Public Health – November, 2013
4. UCSD-School of Medicine Core Curriculum for 2nd year students – November, 2013
5. UCSD-Clinical Research CREST Training Program – November, 2013
6. UCSD-SPPS 261 course for pharmacy students – Winter Quarter, 2013

In addition, trainings for individuals in the justice system and other agencies in California have been conducted:

1. San Diego County Blue Ribbon Commission – February, 2013
2. Los Angeles Alternate Public Defenders Office – August, 2013
3. San Diego County Child Welfare Services – October, 2013
4. San Diego Public Defenders Office – November, 2013

Finally, in September, 2013, we hosted a visiting scholar, Victoria Coathrup, from the UK who is completing her pre-doctoral training with a dissertation on nutrition and alcohol in pregnancy.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Not defined	Uriu-Adams JY, Obican SG, Keen CL. Vitamin D and maternal and child health: overview and implications for dietary requirements. Birth Defects Res C Embryo Today. 2013 Mar;99(1):24-44. PubMed PMID: 23723170.

I. Principal Investigator: Tatiana Foroud, Ph.D. and Peter Hammond, Ph.D.

II. Title of Project: Craniofacial Dysmorphism & Fetal Alcohol Exposure

III. Objectives:

1) Develop a screening tool that will utilize the data from the 3D facial images and support accurate identification of individuals with a high likelihood of alcohol exposure.
2) Recruit and analyze facial imaging data from very young populations to develop a screening tool that accurately identifies high risk individuals for future intervention.
3) Combine face images, neurobehavioral data and brain images to identify common pathways and hence improve diagnosis of prenatal alcohol exposure.
4) Extend existing and develop novel techniques and associated software to cope with demands of larger datasets and more diverse comparison of controls, alcohol exposed and other developmentally delayed subjects while accommodating multiple anatomical images per subject.
5) Extend preliminary genetic studies through collection of DNA samples for new subjects and focused analysis to replicate candidate genes identified in basic science components.

IV. Methods:

We are currently focusing on the collection of a sufficient number of 3D facial images to allow us to perform analyses with the 3DMD camera system. Since our progress report last year, we have collected 1,164 new images and 313 new DNA samples.

Table 1: Summary of data/sample collection through November 1, 2013 (3dMD only)

Site	3D Images (# subjects)[1]	DNA[1]
San Diego	328 (255)	208
UCLA/USC	73 (67)	80
Atlanta	203 (203)	145
Minneapolis	132 (131)	112
Ukraine	47 (34)	0 (no approval)
South Africa (Jacobson)	517 (311)	225[2]
South Africa (May)	37 (37)	0 (no approval)
South Africa (PASS)	2,359 (1219)	0 (collected as part of parent study)
Totals	**3,695 (2,257)**	**770**

[1] Some subjects have now had longitudinal image collection and longitudinal saliva collection (for DNA)

[2] 218 DNA samples also obtained from the mothers and 52 DNA samples obtained from the father. RNA also collected at this site

Biospecimen collection for DNA isolation was initially funded as a supplement to U01 AA014809 but is now budgeted as part of the current grant. DNA is collected as either saliva or a blood sample. The saliva kits are shipped back to Indiana University and extraction of the kits is up to date.

V. Accomplishments and Results:

Specific Aim 1: Develop a screening tool that would utilize the data from the 3D facial images and could be widely used to accurately identify individuals with a high likelihood of alcohol exposure.

Rationale*: Our initial work has focused on a dataset from South Africa that includes individuals between the ages of 3 and 16. We are now expanding the dense surface modeling (DSM) and face signature analyses to develop models (frameworks and algorithms) that can be used more broadly across different populations. To facilitate this work, we will focus on the evaluation of a larger number of individuals with a wider age range as well as a much larger control sample which reflects the diversity of the alcohol-exposed sample. We will also compare the ability of the algorithms to discriminate between those with prenatal alcohol exposure and other dysmorphic syndromes.*

Background

A large number of new 3D images have been reviewed and used to construct dense surface models of face shape. Table 2 below summarizes the number of subjects that have complete data and hence are usable for face shape analysis.

Table 2: Summary of images analyzed (as of November 7th 2013)

Site	Subjects available	Images @ UCL	Complete data	HC[a]	FAS/ PFAS[a]	HE[a]
San Diego	255	234[b]	182	127	5	50
Los Angeles	67	52[b]	53	16	11	26
Atlanta	203	189	85	55	8	22
Minneapolis	131	110[b]	0	0	0	0
Totals	**656**	**585**	**320**	**198**	**24**	**98**

[a] The exposure breakdown in the Caucasian/Hispanic subset: HC=132; FAS/PFAS=18; HE=64; UNK=9
[b] Images sent on 11/8 to UCL: San Diego: 38; Los Angeles: 21; Minneapolis 21

Preliminary results

We have only included images in the current analysis if they have been examined by a member of the Dysmorphology Core and a classification (HC/HE/FAS/PFAS) has been recorded in the repository. Mike Suttie has built a dense surface model of 320 US subjects and has repeated the sequence of analyses previously used (Suttie et al, 2012). The African-American CIFASD subgroup analyzed has only 5 individuals with a FAS/PFAS designation. Two Hispanic individuals with a FAS/PFAS designation have temporarily been included in the Caucasian analysis.

Comparison of means for Caucasian/Hispanic and Cape Coloured cohorts

The normalized means of the FAS/PFAS and HE subgroups are shown below (Fig. 1) and compared to the same means for the South African cohort in the recent Pediatrics paper (Suttie et al, 2012). A comparison of Fig. 1 (A&E) suggests the shortening of the nose in the Cape Coloured FAS/PFAS subgroup does not occur at the same significance level in the Caucasian subgroup. In contrast, the overall reduction in mean face size seems more significant in the

Caucasian FAS/PFAS subgroup. Because the face shape variation dwarfs the philtrum shape variation, the significance level needs to be reduced to 0.5 SD before evidence of philtral smoothing is indicated (Fig. 1 B&F). This is not necessary when the model employs only a philtrum patch. As portrayed, the smoothing of the philtral groove in the Caucasian/Hispanic subgroup occurs by reduction of the philtral pillars closer to the vermillion border of the lip (yellow ring surrounded by green in B), whereas in the Cape Coloured subgroup it is slightly closer to the subnasale and corresponds to "expansion" of the groove (small blue spot surrounded by green in F). These localized differences of significance may simply be due to the smaller size of the Caucasian FAS/PFAS subgroup.

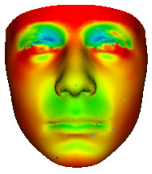
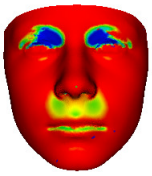
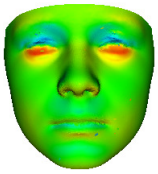
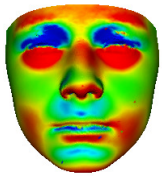
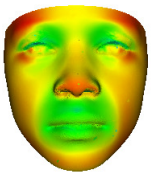
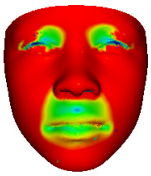
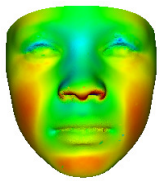
MEAN FACES NORMALISED with regard to 35 CONTROLS	**FAS+PFAS**		**HE**	
	1.5 SD	**0.5 SD**	**1.5 SD**	**0.5 SD**
CAUCASIAN + HISPANIC	(n=18)		(n=64)	
	A 	**B** 	**C** 	**D** 
CAPE COLOURED	(n=48)		(n=75)	
	E 	**F** 	**G** 	**H** 

Figure 1: Summary of Analysis in the Caucasian/Hispanic Subset: (Note: red/yellow under and blue above eyes in Caucasian means in A and C above may be an artifact caused by subjects being encouraged to look upwards rather than at the camera). Red-green-blue denotes surface retraction-coincidence-expansion compared to matched control mean, usually at 2.0 SD unless otherwise stated.

At 1.5 SD, the comparison of the HE subgroups (Fig. 1 C & G) shows overall size comparable with the matched controls in both Caucasian and Cape Coloured cohorts. At 0.5 SD, the Caucasian/Hispanic subgroup mean shows more significant philtral smoothing and once again is closer to the vermillion border (Fig. 1 D & H).

Face signatures

The face signatures of the Caucasian/Hispanic FAS/PFAS subgroup (Figure 2) show considerable reduction in facial growth and/or malar flattening. The third individual on row two has an unusual face with a prominent maxilla and micro/retrognathia as the profile confirms:

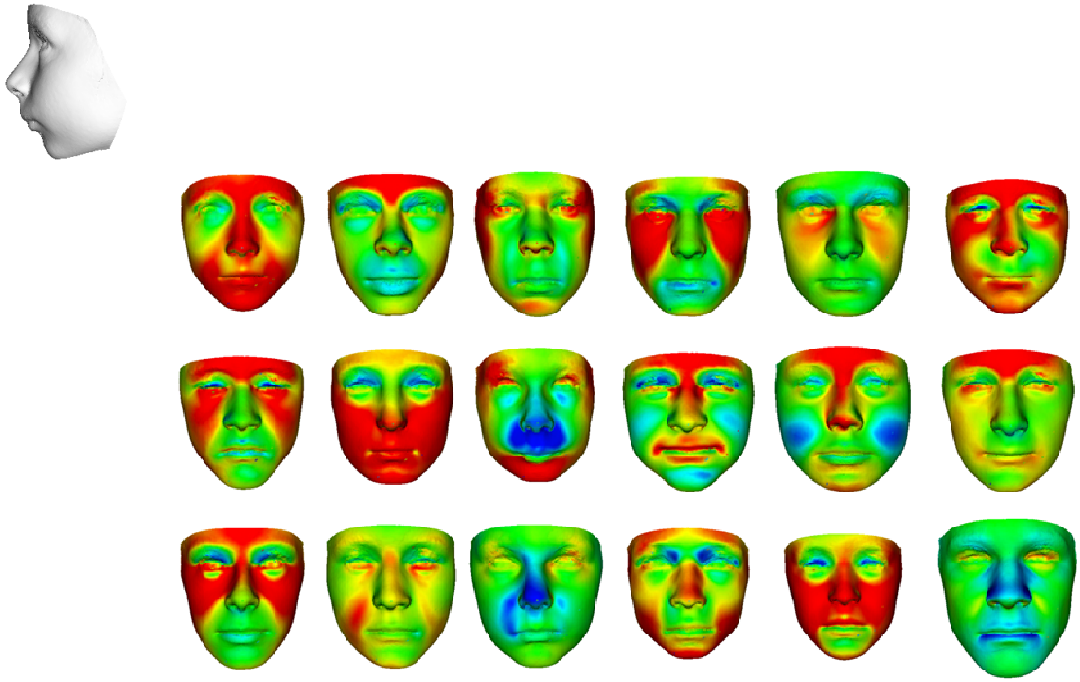


Figure 2 Face signatures (at 2 SD) of Caucasian/Hispanic FAS/PFAS subgroup

The last individual on the last row appears to have normal face size, no malar flattening and from the signature shown in the last item of the last row of Fig. 3, even at 1.0 SD, shows little uniform smoothing of the philtrum. Hence this individual looks somewhat out of place in the FAS/PFAS category. The third individual on the last row similarly has relatively normal face size but a much clearer signal of philtrum smoothing in Fig. 3.

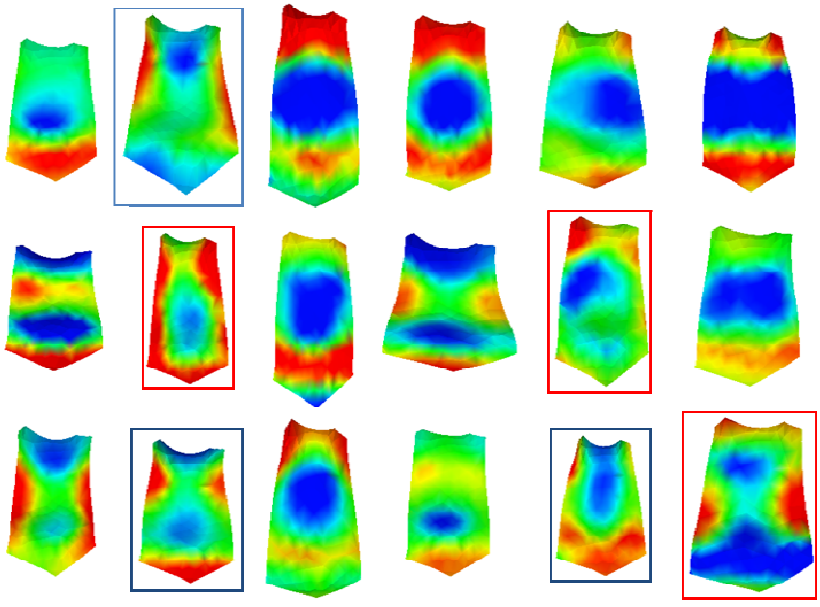


Figure 3: Philtrum signatures of Caucasian/ Hispanic FAS/ PFAS subgroup. Signatures shown at 2 SD unless outlined in blue (1.5 SD) or in red (1.0 SD)

Face classification

Multi-folded classification of face shape using three pattern recognition algorithms (closest mean - CM, linear discriminant analysis - LDA and support vector machines - SVM) was carried out as previously with the Cape Coloured cohort. The small size of the FAS/PFAS group, though, renders the results less reliable than those for the Cape Colored cohort.

Table 3: Multi-folded classification of face shape for HC vs FAS/PFAS

HC versus FAS/PFAS	CM	LDA	SVM
Reduced Face	95%	96%	96%
Thin profile	95%	94%	97%
Philtrum	94%	94%	95%

Nevertheless, it is interesting to note that the mid-facial profile performed at as high a level as the full face and philtrum, as it did for the Cape Coloured cohort.

Discussion and future directions

At the moment, there are insufficient numbers of individuals in the FAS/PFAS category for the results to be publishable. Efforts are already being made to complete the repository entries for about 200 individuals recruited in the USA whose face images have yet to be analyzed. No analysis of African American individuals has been carried because of the extremely low number of individuals with a full data description and in the FAS/PFAS category.

Some cluster analysis of the US Caucasian/Hispanic data has been completed and a number of signature graphs have been generated along the lines of the analysis of the Cape Colored cohort with a view to linking facial dysmorphism and cognitive impairment. However, the small size of the FAS/PFAS subgroup renders the results even less reliable than the multi-folded classification. These will be completed more fully once the dataset is expanded.

Specific Aim 2: Recruit and analyze facial imaging data from very young populations to develop a screening tool that accurately identifies high risk individuals for future intervention.

Rationale*: Based on the results of cross species analyses, we hypothesize that the facial features that distinguish those who are alcohol exposed from those who were not, differ across the lifespan and it may not be possible to develop a single model that can accurately discriminate the groups at all ages. To test this hypothesis, we collecting facial images from 1,200 South Africa PASS subjects at 1 month and 12 months and are using both DSM and face signature analysis methods similar to those in Aim 1. We will compare results in the South Africa cohort with those obtained in the Ukraine sample from which we will obtain images at 6 and 12 months. We anticipate that the novel algorithms developed in this younger cohort will be very valuable in identifying those at high risk for future intervention trials.*

Background

We have recently completed the collection of images at the 1 month and 12 month time points in the PASS subjects. Initial analyses were performed without the benefit of any alcohol exposure data which undermined the normalization process since no unexposed category was

revealed by the PASS consortium. Nevertheless, teleconference presentation and discussions, and also a face-to-face presentation by Michael Charness, convinced the PASS consortium to reveal the identity of a small but useful number (100) of unexposed individuals so that they could act as controls for the normalization process. When this information was made available, the analysis was repeated and preliminary results were presented by Peter Hammond at the PASS face-to-face meeting held at Bethesda in September 2013.

Table 4: Summary of South African data analyzed as of November 7th 2013

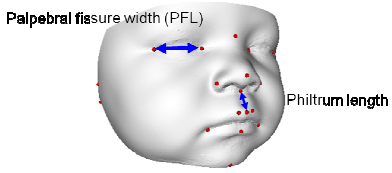
South Africa Sites	**Subjects available**	**Images @ UCL**	**Total in models**
Jacobson	311	210[a]	192
May	37	37	0
PASS	1,219	900	246
Totals	**1,567**	**1,147**	**438**

[a] 90 images being processed currently at IU to be sent to UCL

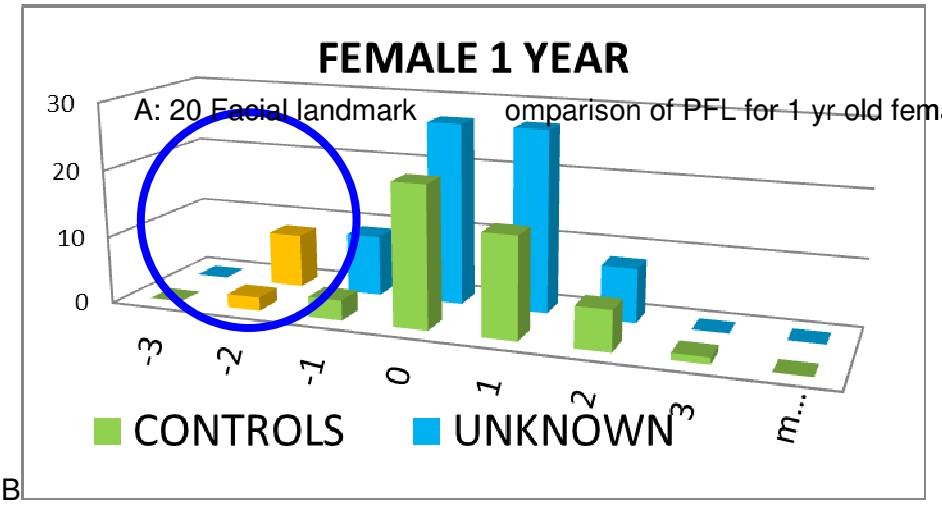
(Fig. 4B) in order to identify outliers (within blue circle).

Preliminary results

Dense surface models of the face and philtrum have been generated for 246 PASS infants of whom 91 are known to have had no or minimal prenatal alcohol exposure. A typical set of 20 landmarks was used (Fig. 4A) enabling a dense surface correspondence to be induced and specific measures to be derived e.g. palpebral fissure length (PFL) in female infants at 1 year (Fig. 4B) in order to identify outliers



A



B

Figure 4: A: 20 Facial landmarks; B: C

Face signature graphs were constructed as an alternative way to identify outliers with features compatible with prenatal alcohol exposure. For example, Fig. 5 shows a face signature graph for 1 month old babies with preterm individuals highlighted in red squares.

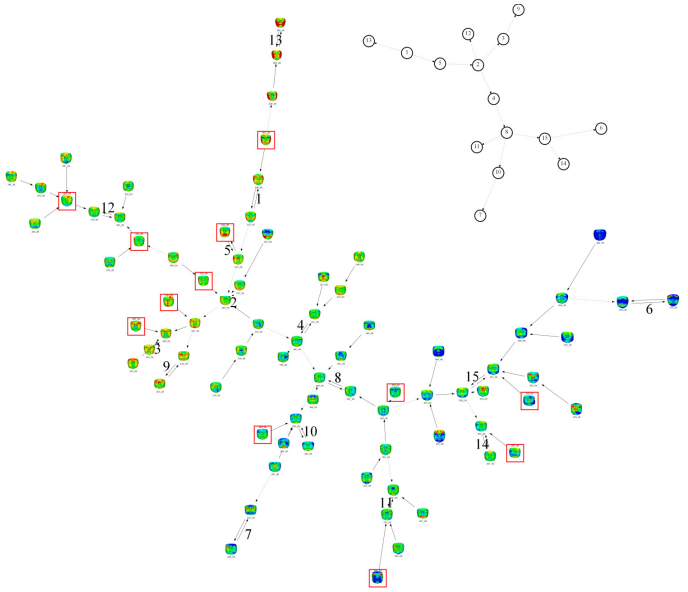


Figure 5: Face signature graph of 1 month old babies Facial growth range: “smaller” faces at left and top and “larger” to the right and bottom.

Similar signature graphs were generated for the philtrum shape of those infants declared as unexposed (controls) to determine which if any might be classified as having a smooth philtrum. Indeed this was the case for a handful as indicated by enclosing ellipse.

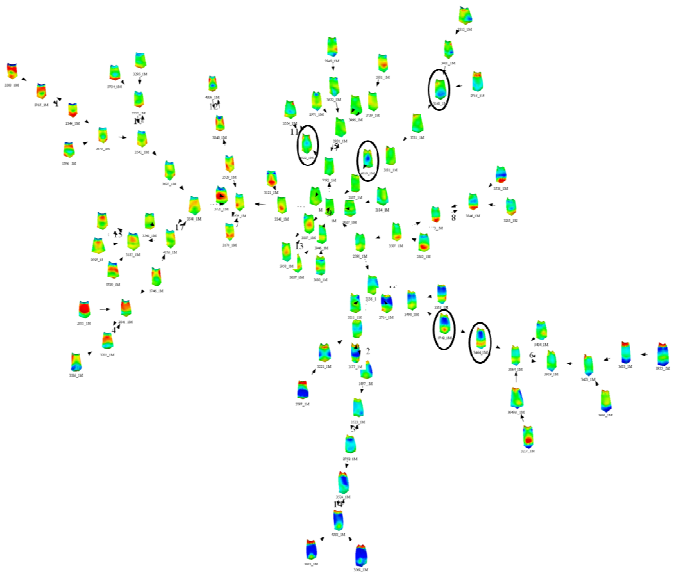
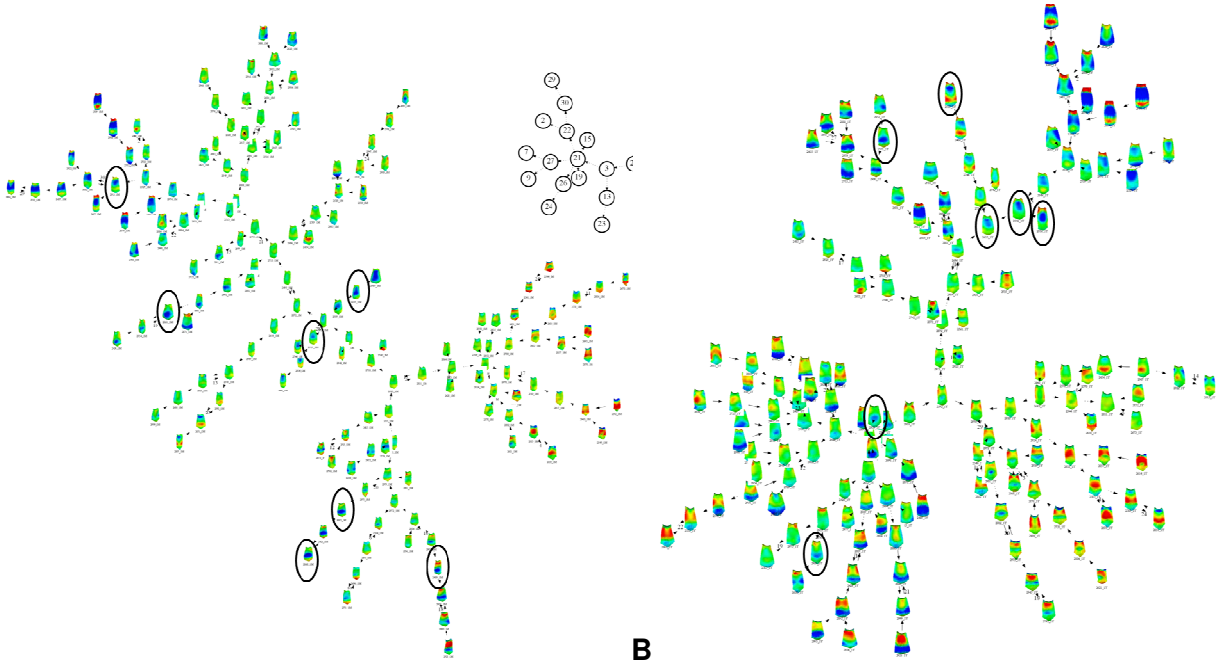


Figure 6: Philtrum signature graph of 91 unexposed infants at 1 month

It will be important to double check the exposure status of these children prior to the follow on analysis of the complete dataset. A similar philtrum signature graph for all 1 month olds with cryptic exposure status can be used to identify a subgroup who have significant philtral smoothing at one month (Fig. 7A) or at one year (Fig. 7B).



A B

Figure 7: Philtrum signature graphs. A. 1month; B. 1 year old infants with cryptic alcohol exposure status

We still need to decide what combination of palpebral fissure length, philtrum smoothness, face shape and facial growth will be used as the final criteria for selecting candidates to be classified in terms of facial characteristics compatible with prenatal alcohol exposure (Fig. 8).

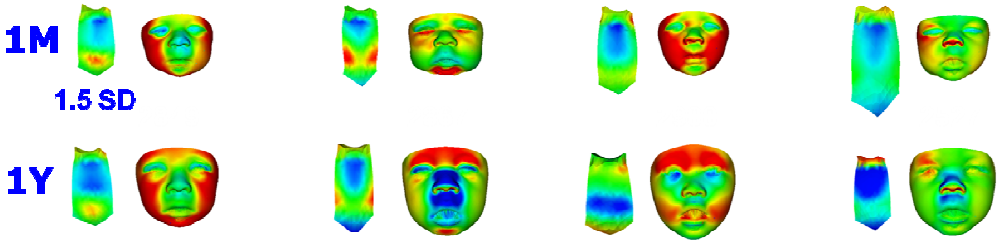


Figure 8 Paired face/philtrum signatures at 1m and 1 yr of several infants with cryptic alcohol exposure status who have facial characteristics compatible with prenatal alcohol exposure.

Discussion and future directions

It has been agreed with the PASS consortium that the remaining 700+ infants will be analyzed in a similar manner to the 246 analyzed so far. When the CIFASD face analysis team is confident of predictions of exposure/lack of exposure, a list of annotated IDs will be sent to DM-STAT so that sensitivity and specificity calculations can be determined. At that point, there will need to be joint discussions concerning the possibility of publication. At the PASS September

face-to-face meeting and in a recent teleconference call between Amy Elliot, Tatiana Foroud and Peter Hammond, the possibility of the PASS consortium revealing more detailed analysis of alcohol exposure data such as timing was discussed.

During a recent trip to South Africa, a new cohort of 72 babies was imaged, not with the PASS consortium, but as part of a new study led by Drs. Joseph and Sandra Jacobson. These babies all have data regarding prenatal alcohol exposure. Additional assessments will be performed over the coming year and these data will be combined with the imaging data to develop new analyses. Results from these analyses will complement ongoing analyses of babies of a similar age that are ongoing in the PASS sample and will begin once data collection is ongoing in the Ukraine.

Specific Aim 3: Combine face images, neurobehavioral data and brain images to identify common pathways and hence improve diagnosis of prenatal alcohol exposure.

Rationale: Based on our initial results, optimal discrimination between controls and those who have been alcohol exposed but do not meet criteria for FAS or PFAS demands the inclusion of non-facial measures. Within CIFASD, data from neurobehavioral testing as well as brain imaging is available and we will develop models that combine specific neurobehavioral measures and 3D facial images to improve our ability to identify those with prenatal alcohol exposure.

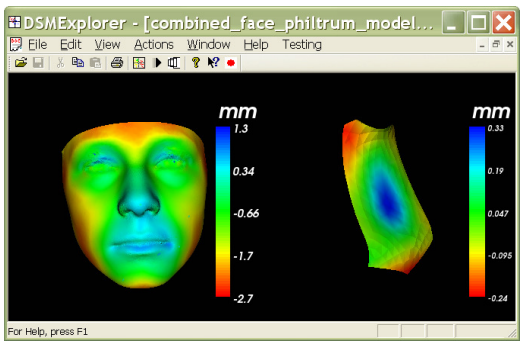
Background

The subjects seen in South Africa in collaboration with Drs. Sandra and Joseph Jacobson have now had facial imaging collected at 2 time points (using the 3dMD system) and many also have brain imaging data. Data has recently been shared by the Jacobson's to allow the use of the brain imaging data with the facial images.

As far as the CIFASD dataset is concerned, there are 330 records of MRI images on the repository of which 125 are obtainable and of those we have 63 matching 3D face images. There are 52 brain images from Philip May which have 37 associated 3D face images available.

Preliminary results

Mike Suttie and Peter Hammond have worked closely on the design of a new version of the model construction and model viewing modules of the UCL face analysis so that sets of paired shapes can be analyzed for shape correlations. The obvious candidate is a set of paired 3D face and MRI images but other possibilities are also being pursued: paired face and philtrum; paired face and skull; paired parent and child faces. After a considerable effort, Mike Suttie has produced a prototype that is able to combine two separate shape models into one, to visualize the PCA modes of the correlated shape variation of the pairs and to construct mean and normalized paired shapes. Some small models have been constructed for face-philtrum (Fig. 9A) and face-corpus callosum (Fig. 9B) pairs

A 

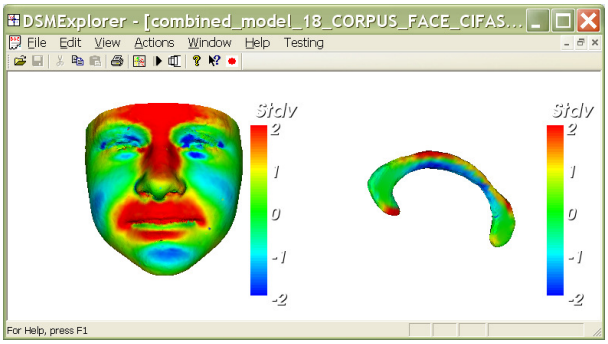
B 

Figure 9: **Combined model analysis interface**.
A. face-philtrum pairs; B. face-corpus callosum pairs

Discussion and future directions

The prototype still has some bugs to iron out and it still does not produce animations or signature graphs. So these additional features are future work. Some additional potential applications of paired models have arisen outside the CIFASD consortium. For example, Peter Hammond has become a second supervisor of a young surgeon undertaking a PhD in a study aiming to tissue engineer a customized replacement zygomatic arch for children with Treacher-Collins syndrome. A paired model of 3D face shape and anterior skull will be developed so that an affected child can be matched to an age-matched average zygoma shape which will be 3D printed to form a scaffold for stem cell culture and subsequent bone growth. Mike Suttie's paired model version of the shape analysis software will be an important tool in this exciting project.

Specific Aim 4: Extend existing and develop novel techniques and associated software to cope with demands of larger datasets and more diverse comparison of controls, alcohol exposed and other developmentally delayed subjects while accommodating multiple anatomical images per subject

Rationale: *Existing software tools analyze hundreds of 3D images simultaneously subject to limitations of a 32-bit architecture. The aims above will require analyses of thousands of images to support broader age ranges, integration of non-facial data, ethnic comparison and comparison with developmental delay of a genetic or non-alcohol related origin. We will migrate existing software to a 64-bit architecture and develop new analytical approaches to take advantage of increased computational power.*

Progress

The migration of the UCL face analysis software from 32 bit to 64 bit is complete. Small "bugs" have arisen occasionally but all have been easily dealt with. Last year's progress report described how a model was constructed for the philtrum patch of 4,747 3D face images of the ALPSAC datset. We have now been able to extend model construction to the faces of the same image collection and are confident that any limitations on size of dataset we can analyze is now limited only by the RAM available on the computer used. We will continue to test the implementation e.g. with a multi-syndromic model for differential diagnoses of fetal alcohol syndrome and overlapping genetic conditions.

Specific Aim 5: Extend preliminary genetic studies through collection of DNA samples for new subjects and focused analysis to replicate candidate genes identified in basic science components.

Rationale: *A goal of CIFASD is to identify those at greatest risk for physical and behavioral abnormalities following prenatal alcohol exposure. Preliminary data suggests that there may be DNA polymorphisms that have differential effects on facial development in the presence or absence of alcohol prenatally. We will continue to explore this hypothesis using candidate genes nominated from other projects in CIFASD.*

Background

Data from the Illumina OmniExpress array was generated in a sample of 240 CIFASD subjects selected based on the extent of data available as well as the availability of DNA. Initially, analyses were performed using anthropometric measurements obtained from the facial image. More recently, Dr. Peter Hammond generated PCA measurements for each subject with genotypic data. The PCA mode values included measures of the reduced (smaller) facial surface as well as the philtrum.

Preliminary results

Analyses were recently completed to review the evidence of association with SNPs genotyped in specific candidate genes nominated from studies performed by Dr. Michael Charness. The genes included: *RPS6KA1, RAF1, FYN, BRAF, LYN, MAP2K1, CSNK2A2, BCAR1, MAP2K3, YES1, CSNK2A1, SRC*. Analyses were performed in the European American subset, using the first 6 principal components provided for the reduced facial surface and the philtrum. All analyses included age and gender as covariates in a linear regression model. The focus of the analysis was to identify SNPs that had a differential effect with prenatal alcohol exposure. Therefore, the focus of the analysis was to identify significant SNP x alcohol exposure interactions.

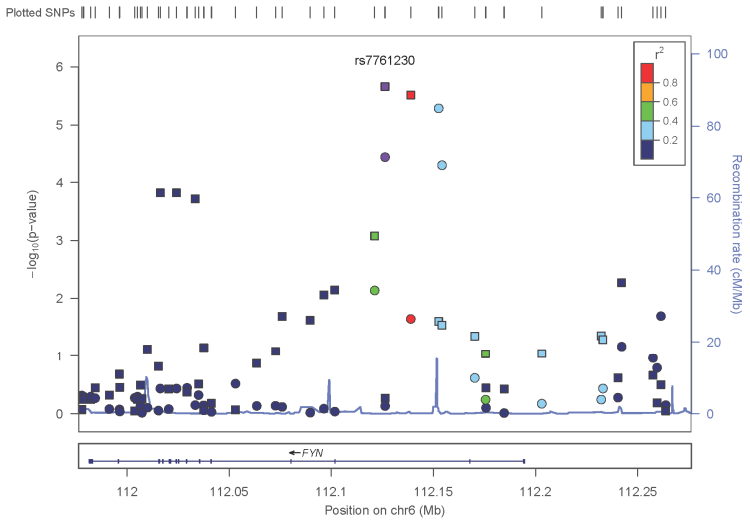


Figure X: Association results in *FYN* on chromosome 6. The circles represent p-values for the interaction term when using PC1, a measure of overall size, or growth of face. The squares represent the p-values for the interaction term when using PC2, a measure of facial width and length (oval vs square). The shading of each symbol represents the extent of linkage disequilibrium with the most significant SNP, rs7761230.

Results in *FYN* suggest that there may be an association with measures of reduced facial surface. The most significant results are supported by several SNPs. Of note, the SNPs providing evidence of association are 5' of the gene.

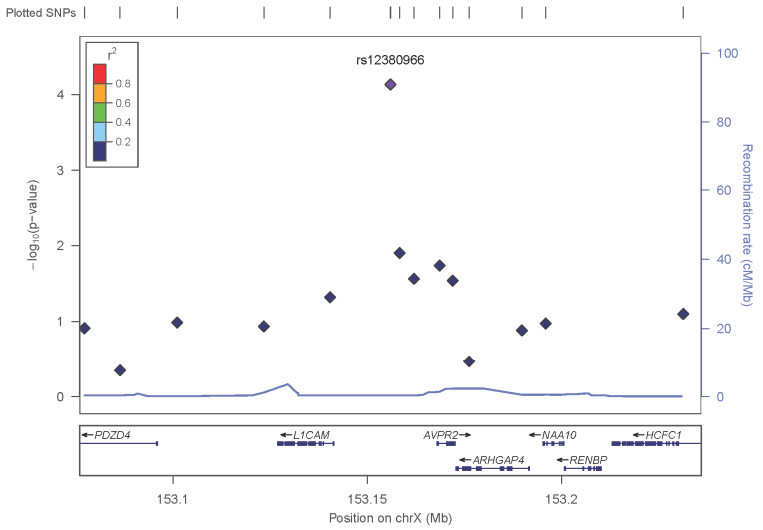


Figure X: Association results in *L1CAM* on chromosome X. Results represent p-values for the interaction term when using PC6 for the reduced facial surface measures, depicting hypertelorism and mandibular retraction. The shading of each symbol represents the extent of linkage disequilibrium with the most significant SNP, rs12380966. Of note, the other SNPs in the region are not in linkage disequilibrium with the most significant SNP and do not provide additional evidence of association.

L1CAM is a quite small gene and few SNPs were genotyped in or around this gene. The most significant SNP with an interaction (SNP x alcohol exposure) p-value is located in the region 5' of *L1CAM*, between *L1CAM* and *AVPR2*. The frequency of the minor allele at the SNP rs12380966 is only 11%. Therefore, there are few individuals who have inherited the minor allele. Although this result seems promising, further evaluation in a larger sample is required.

Discussion and future directions

The initial results exploring candidate genes has been reasonably successful. We will continue to review results in genes nominated by our CIFASD collaborators. We will explore analyses utilizing additional phenotypes related to reduced facial surface and will also explore these results in samples of other race/ethnicity. We anticipate as additional DNA samples accrue in this study that we will apply to CIDR for additional genotyping to expand the scope and power of the sample.

VI. Discussion:

VII. Interrelation with Aims of the Consortium and Other Projects:

Face-Brain Subgroup **–** Currently, there are 567 individuals with both 3D image and neurocognitive data. There are 136 individuals with both 3D image and brain volume data (MRI). There are 121 individuals with data from all three domains: 3D image, neurocognitive, and brain volume data (MRI). Hypotheses are being developed which will explore the impact of alcohol exposure on these various domains in this growing sample.

Coordination with the Bioinformatics Core – The Bioinformatics Core members have created a new interface for uploading the data so that xml and mdl files can be uploaded simultaneously. The x,y,z, coordinate data are now a part of the data base. The new interface accommodates longitudinal data. The Bioinformatics Core has also improved the rate at which images can be uploaded to the central repository, and is working to implement a method to track in the Central Repository. Images obtained for CIFASD subjects recruited through a CIFASD site are included.

The SNP genotypic data is available in the central repository. In addition, principal components generated from the SNP genotypic data are available for CIFASD investigators to use as race covariates in analysis. A new variable was added to the central repository that indicates if the subject has a DNA sample available.

Coordination with Dysmorphology Core - All subjects were seen by a member of the Dysmorphology Core and assigned a diagnosis of FAS, deferred, or no FAS.

Coordination with mouse project – Mike Suttie has used the atlas based software provided by UCL colleagues to process a collection of mouse MRI images for collaborators at UNC. When they are transferred to Kathy Sulik's team and segmentation is complete, we will continue the collaborative comparison of neurofacial effects of timing of exposure.

Coordination with pilot projects – DNA samples were sent to Dr. Dipak Sarkar for use in his pilot study. This included 87 samples from 47 controls, 27 exposed non-FAS, and 13 FAS. Members of this core assisted in the analysis of the resulting data.

Coordination with Administrative Core/NOFAS – This year, the members of this project have developed materials and videos which can be used to educate others about the importance of facial imaging in the development of improved methods to identify children exposed to alcohol prenatally. Video links were provided to the Administrative core that show how the 3DMD camera is operated and discusses the significance of the work accomplished to date.

VIII. Plans for the Next Year:

Sites for data collection –We will continue to collect images in San Diego, Atlanta, Los Angeles, Minnesota and the Ukraine. We are exploring the collection of data at the US PASS site in South Dakota. We are also developing a collaboration that would couple the collection of additional images from older individuals the PASS cohort in South Africa with an fMRI study.

Data collection – We will continue to collect both 3D facial images and saliva for DNA collection from all participating CIFASD sites. We do not anticipate any modifications in our data collection protocols.

Data analysis – We will continue to distribute images to all project collaborators and facilitate with the analysis of data.

Algorithm development and software implementation – The sections above have outlined the ongoing nature of this and the necessity for progressive development, feedback from clinical and other CIFASD colleagues and subsequent improvement underpins the next year.

IX. Publications:

1. Lipinski RJ, Hammond P, O'Leary-Moore SK, Ament JJ, Pecevich SJ, Jiang Y, Budin F, Parnell SE, Suttie M, Godin EA, Everson JL, Dehart DB, Oguz I, Holloway HT, Styner M, Johnson GA, and Sulik KK. Stage-specific ethanol exposure causes unique face-brain dysmorphology patterns in a mouse model of fetal alcohol spectrum disorder. *PLoS ONE*, 2012;7(8):e43067. doi: 10.1371/journal.pone.0043067. Epub 2012 Aug 22. PMID: 22937012

2. Suttie M, Foroud T, Wetherill L, Jacobson S, Jacobson JL, Molteno CD, Hoyme HE, Hammond, P. Facial dysmorphism across the fetal alcohol spectrum, *Pediatrics*, 2013 Mar;131(3):e779-88. doi: 10.1542/peds.2012-1371. Epub 2013 Feb 25. PMID: 23439907

3. McCarthy N, Wetherill L, Lovely CB, Swartz ME, Foroud TM, Eberhart JK. Pdgfra protects against ethanol-induced craniofacial defects in a zebrafish model of FASD. Development. 2013 Aug;140(15):3254-65. doi: 10.1242/dev.094938. PMID: 23861062

X. Posters and Presentations:

April 2013 Invited presentation at Italian Medical Genetics conference, Bologna, Italy (Peter Hammond)

April 2013 UNC-Chapel Hill, Chapel Hill, NC. "What's behind the screen: using zebrafish to identify gene/ethanol interactions" (Johann Eberhart)

April 2013 North Carolina Central University, Durham, NC. "What's behind the screen: using zebrafish to identify gene/ethanol interactions" (Johann Eberhart)

May 2013 Stower's Institute for Medical Research, Kansas City, MO, "Generating variation in craniofacial disease"

June 2013 Seminar at Centre for Gene Function, Oxford University, UK (Peter Hammond)

July 2013 8th European Zebrafish Meeting, Barcelona, Spain. "What's behind the screen: using zebrafish to identify gene/ethanol interactions" (Johann Eberhart)

Sept 2013 Face-to-face meeting of PASS Network, Bethesda, USA (Peter Hammond)

October 2013 UT Austin SAGE (continuing education group for senior citizens), Austin, TX, "Fetal Alcohol Spectrum Disorders" (Johann Eberhart)

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
In process at NIHMS	McCarthy N, Wetherill L, Lovely CB, Swartz ME, Foroud TM, Eberhart JK. Pdgfra protects against ethanol-induced craniofacial defects in a zebrafish model of FASD. Development. 2013 Aug;140(15):3254-65. PubMed PMID: 23861062.
Complete	Suttie M, Foroud T, Wetherill L, Jacobson JL, Molteno CD, Meintjes EM, Hoyme HE, Khaole N, Robinson LK, Riley EP, Jacobson SW, Hammond P. Facial dysmorphism across the fetal alcohol spectrum. Pediatrics. 2013 Mar;131(3):e779-88. PubMed PMID: 23439907; PubMed Central PMCID: PMC3581841.

Interim Progress Report
April 1, 2013 - November 11, 2013

I. Principal Investigator

PI: Sarah Mattson

Site PIs: Julie Kable (Emory), Jeff Wozniak & Chris Boyes (University of Minnesota), Elizabeth Sowell (USC)

Other Investigators/consultants: Ed Riley, Claire Coles

II. Title of Project

A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders

III. Objectives

The overall objective of our project is to develop and implement clinically relevant and feasible measurement tools to accurately identify alcohol-affected children across a broad range of ages. In keeping with this objective, the specific aims of our project are:

1. Use existing data to develop a tiered or hierarchical approach to identification of affected cases.
2. Test the specificity and sensitivity of the model developed in Aim 1 in children ages 10-16.
3. Test the utility of the model in younger children, ages 5-7.
4. Targeted assessment of memory function.

IV. Methods

The overall design of Phase III is a cross-sectional study of three subject groups (AE, Controls, and Contrast) and two age groups (5-7 and 10-16 years). These six groups (3 exposure groups x 2 age groups) are assessed with a neuropsychological test battery that includes measures that were shown to be effective in differentiating AE from the CON and ADHD groups in Phase II and additional measures targeting the cognitive domain of memory. The contrast group is a heterogeneous, clinically-referred group of non-exposed children. Subjects are tested at 4 sites using the same neuropsychological test battery.

V. Accomplishments and Results

During the current funding period (since 6/1/2013), we have made considerable progress in several areas.

1. Manuscript Preparation. Using data from CIFASD II data collection, we published 4 papers in 2013, addressing our aims of determining the nature and specificity of the neurobehavioral profile of FASD. Three of these papers have been previously described. The fourth paper (Glass et al., 2013) demonstrated that unlike our behavioral studies, the combination of alcohol exposure and ADHD does not result in exacerbated deficits. Children with alcohol exposure and ADHD did not differ from those with exposure but no ADHD. Further, there were greater deficits on specific outcomes (verbal comprehension and perceptual reasoning) in children with alcohol exposure (regardless of ADHD) than in unexposed children with ADHD. This paper is in “early view” in Neuropsychology.
2. Data Analysis
    a. *Previous aims*. We continue to analyze data relating to our previous aims and have 4 papers under review or about to be submitted. For example, we submitted a paper addressing the interaction between alcohol exposure and a diagnosis of ADHD on

adaptive behavior (Paper under review #1, Ware et al.). Results indicated that, as found previously, both prenatal alcohol exposure and ADHD increase adaptive behavior deficits in all domains. However, these two factors interact to cause the greatest impairment in children with both prenatal alcohol exposure and ADHD for communication abilities. This paper is a companion to the recently accepted paper by Glass et al. (2013, described above). We have also recently submitted a paper demonstrating that parent reports are consistent with direct laboratory measures of attention but not hyperactivity (Paper under review #2, Glass et al.). We further examined this relation by examining parent ratings and laboratory measures of executive function in children with and without alcohol exposure and with and without ADHD. As in Glass et al., 2013, the results supported compounded behavioral, but not neuropsychological, effects in alcohol-exposed children with ADHD. Further, parent reports were not correlated with laboratory performance in the clinical groups. This paper (Paper under review #3, Nguyen et al.) was just submitted for publication. Finally, we followed up on our previous studies of the neurobehavioral profile of alcohol exposure by examining whether multiple profiles exist with in the alcohol-exposed population. For this study, we examined parent ratings of behavior (our previous profile papers used neuropsychological test data). Results indicated 3 profiles within the alcohol-exposed group, characterized as average-, intermediate-, and clinical-level profiles of behavioral problems. We then tested whether the resulting profiles differed on laboratory executive functioning tests. Profiles differed on switching conditions of the Verbal Fluency and Color-Word Interference subtests. These results indicated within-group variability, confirming that alcohol-exposed subjects are not equally affected. Inhibition and cognitive flexibility were identified as targets for behavioral remediation. This paper (Paper under review #4, Graham et al.) was just submitted for publication.

b. *Current aims*. We are continuing to work on our current Aim 1. Since the previous updates regarding this project, demographics have more specifically been analyzed (**Table 1**). Subsequently, the first step in our analysis was to examine correlations between behavioral criteria (BRIEF, DISC, VABS, SCT, CBCL, DBD) and the

Table 1. *Demographic Information for children with and without prenatal alcohol exposure*

Demographic Variable	Alcohol-Exposed (*n* = 147)	Non-Exposed (*n* = 238)
Site [*n* (%)]*		
Albuquerque	12 (8.2)	43 (18.1)
Atlanta	30 (20.4)	38 (16.0)
Los Angeles	28 (19.0)	21 (8.8)
Plains States	22 (15.0)	30 (12.6)
San Diego	55 (37.4)	106 (44.5)
Age [*M* (SD)]	12.47 (2.39)	12.07 (2.63)
FSIQ [*M* (SD)]*	83.88 (16.73)	102.87 (16.90)
Sex [*n* (% Female)]	63 (42.9)	87 (36.6)
Race [*n* (% White)]*	74 (50.3)	119 (50.0)
Ethnicity [*n* (% Hispanic)]*	15 (10.2)	42 (17.6)
Handedness [*n* (% Right)]	126 (85.7)	218 (91.6)
ADHD [*n* (% Diagnosed)] *+	86 (58.5)	94 (39.5)
FAS [*n* (% Diagnosed)]*	40 (27.2)	0 (0)

* Significant at a rate of $p < .05$

+ Presence of ADHD was assessed using the Computerized Diagnostic Interview Schedule for Children-Fourth Edition

presence of prenatal alcohol exposure (yes/no). After conducting correlations and intercorrelations, variables that would be analyzed further included 19 items from dysmorphology, neuropsychological, behavioral, and 3D facial imaging data (**see Table 2**). *Children with FAS were not included in the analyses in order to specifically target the characteristics that will identify non-dysmorphic alcohol-exposed children.*

Two logistic regression analyses were conducted: (1) comparing alcohol-exposed children with ADHD (AE+; *n* = 84) and non-exposed children with ADHD (ADHD; *n* = 90) and (2) comparing alcohol-exposed children without ADHD (AE-; *n* = 38) and non-exposed typically developing children (CON; *n* = 121). All variables from **Table 2** were included in each logistic regression analysis, which allowed for the model to account for all of the variance from these variables contributing to group discrimination. Further, demographics (race, ethnicity, site, and IQ) were included in the model, serving as covariates and allowing for a more accurate reflection of the remaining variance accounted for primarily by the neuropsychological, behavioral, dysmorphology, and 3D imaging data.

Table 2. *Remaining 19 variables after correlational and inter-correlational analyses*

Variable	Correlation
Dysmorphology	
Campodactyly	r = -.250
Difficulty with pronation/supination of elbows	r = -.206
Ptosis*	r = -.151
Neuropsychological	
WISC Similarities	r = .462
WISC Arithmetic Processing	r = .457
WISC Working Memory Composite	r = .394
WISC Perceptional Reasoning Comprehension	r = .389
Verbal Fluency: Switching Total Correct	r = .380
WISC Processing Speed Composite	r = .364
Verbal Fluency: Letter Fluency Total Correct	r = .362
WISC Picture Concepts	r = .356
WISC Letter Span Rhyming Total Correct	r = .355
WISC Digit Span Forward Total Correct	r = .349
Verbal Fluency: Category Fluency Total Correct	r = .330
Behavioral	
BRIEF Inhibition T-score	r = -.482
CBCL School Competence	r = .467
VABS Coping Skills Composite	r = .458
3D Facial Imaging	
Tragion right	r = -.211
Right Palpebral Fissure	r = .210

Note: All correlations were significant at the $p < .001$ level except Ptosis ($p = .008$)

Based on the first analysis, the following variables were able to significantly distinguish AE+ and ADHD: campodactyly, WISC Similarities, WISC Picture Concepts, D-KEFS Verbal Letter Fluency, BRIEF Inhibition T-score. The AE+ subjects were more likely to have campodactyly present, perform significantly poorer on the neuropsychological measures, and have significantly poorer reported inhibition according to the BRIEF in comparison to the ADHD subjects.

Based on the second analysis, the following variables were able to significantly distinguish AE- and CON: WISC Arithmetic Processing Approach: Part B and WISC Perceptual Reasoning.

Two more logistic regression analyses were conducted: (1) AE- versus ADHD and (2) AE+ versus CON. This was done to see if the variables found in the main analyses were specific to discriminating those groups, or if they would simply discriminate these alcohol-exposed subjects from any non-exposed subject. **Table 3** shows the final variables determined to discriminate groups (i.e., variables that were not overlapping in these subsequent logistic regressions). Final logistic regressions were conducted with the final set of variables found to discriminate groups and obtain classification accuracy rates (**Tables 4 and 5**). One issue that we are currently working to understand and improve is the lower classification rates of the alcohol-exposed subjects (both groups). It is not surprising that the AE+ and ADHD groups are more difficult to discriminate, however the low classification rate of the AE- group suggests the high variability among this clinical group that is likely making it difficult to separate some of the AE- subjects from the CON subjects in these analyses.

The CART technique we described previously has not been pursued, as it did not allow for manipulation of specified variables in order to meet the main goal, which is to increase the accuracy of clinical identification for children with prenatal alcohol exposure by assessing performance on specific variables. Rather, it would often choose one variable to distinguish groups and throw out others. However, the use of one variable is unlikely to result in high accuracy when discriminating alcohol-exposed children. Our current methods are successful in identifying a small number of variables that can be used to successfully discriminate groups.

Table 3. *Non-overlapping variables of discrimination*

Alcohol-exposed with ADHD versus non-exposed with ADHD	Alcohol-exposed without ADHD versus typically developing controls
WISC Picture Concepts	WISC Arithmetic
Campodactyly	WISC Perceptional Reasoning Composite
D-KEFS Verbal Letter Fluency	

Table 4. *Logistic regression discriminating AE+ and ADHD using WISC Picture Concepts and Campodactyly*

Classification Table[a]

	Observed		Predicted		
			AE+ vs. ADHD		Percentage Correct
			AE+	ADHD	
Step 1	AE+ vs. ADHD	AE+	55	25	68.8
		ADHD	24	46	65.7
	Overall Percentage				67.3

a. The cut value is .500

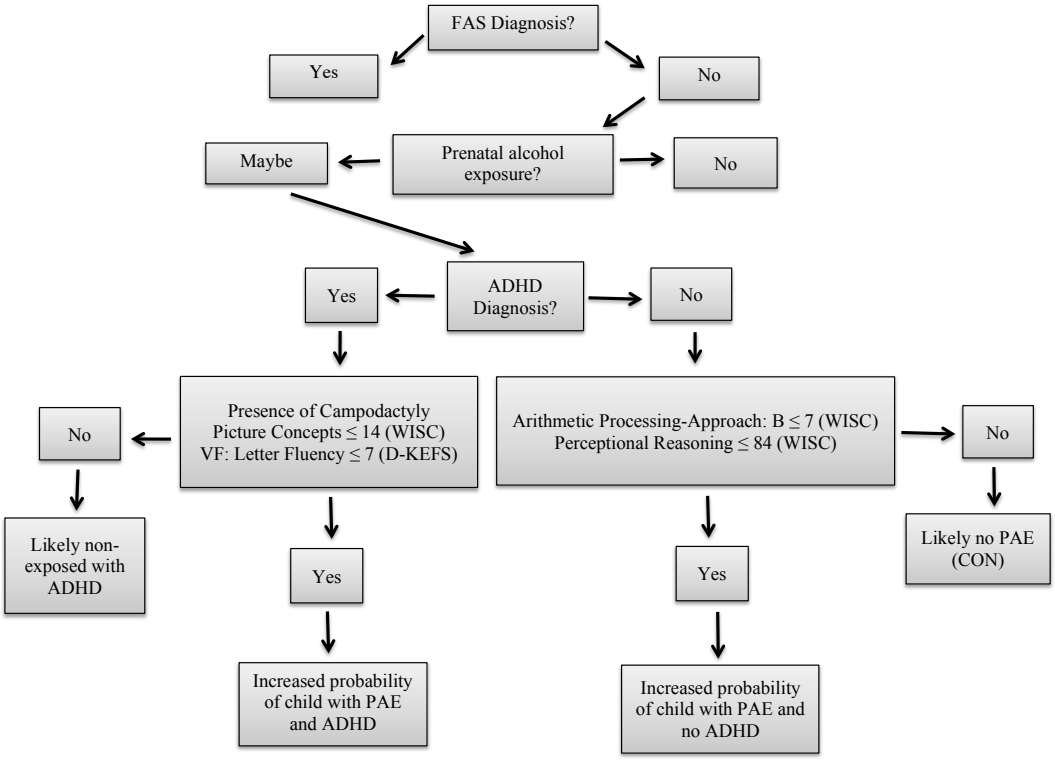
Table 5. *Logistic Regression discriminating AE- and CON using WISC Arithmetic PA: Part B and WISC Perceptual Reasoning*

Classification Table[a]

Observed		Predicted		
		AE- vs. CON		Percentage Correct
		AE-	CON	
Step 1 AE- vs. CON	AE-	26	13	66.7
	CON	5	138	96.5
	Overall Percentage			90.1

a. The cut value is .500

Figure 1: *Simple conceptualization of final product to be clinically utilized for identification of alcohol-exposed children without FAS*



3. Data Collection. Data collection is active at all sites. Since the initiation of data collection (November and December of 2012 as per our original proposeal), we have tested 253 subjects, which represents 28.75% of our overall goal for phase III. This considerable progress was made even when our initial project year was shortened and our budget was decreased. In addition, these subjects represent the subjects that are actually in the database and have been considered "complete" (see below).

4. Database development. While we previously reported the completion of the database and development of user-guides and data dictionaries, since our previous progress report, we developed, with the assistance of the informatics core, an automatically generated tally of complete participant data that informs monthly conference calls as to each site's progress. See below for sample tally. These tallies represent "complete" subjects entered into the central repository and may differ from the number tested.

SITE	Total Subjects	5-7 AE	5-7 Control	5-7 Contrast	5-7 Total	10-16 AE	10-16 Control	10-16 Contrast	10-16 Total
SMS	86	8	12	5	25	26	24	11	61
JWM	87	6	12	2	20	28	22	17	67
CCA	55	13	5	7	25	10	14	6	30
ESL	25	0	0	0	0	15	10	0	25
TOTAL	253	27	29	14	70	79	70	34	183

VI. Discussion and Significance

The neurobehavioral project is well underway and is thus far, meeting our project goals. The biggest hurdle was initiating data collection on time and that was accomplished last year. We continue to collect and analyzed data and are moving forward relatively smoothly. Four papers have been published and 4 are under review. We continue to make progress on our first specific aim and hope to complete that during the remainder of the current funding year.

VII. Interrelation with Aims of the Consortium and Other Projects

This project is strongly integrated with the rest of CIFASD in several ways. On the site level, four sites are participating in this component. On the project level, subjects tested for Phase III will be assessed as part of the 3D facial imaging (T. Foroud) and brain imaging (E. Sowell) projects as well as the genetics developmental project (T. Foroud) and data from all projects will be analyzed. The Phase III neurobehavioral project also dovetails with the projects in the Ukraine (C. Chambers) that involve preschool age children (ages 3-4). When old enough, these subjects could be tested and therefore act to validate the resulting "young" profile. Finally, on the level of the cores, this project integrates with the Dysmorphology core (K. Jones), the Informatics core (W. Barnett), and the Administrative core (E. Riley). All children will receive a standardized dysmorphology examination, which will provide diagnostic information, aid subject classification, and provide data for the aims of the Dysmorphology core. We are working closely with the Informatics core in development of a database as well as the data input and output tools in the central repository (see above). These tools will facilitate uniform and validated data entry across sites and provide the basis for all statistical analyses. Finally, our specific aims will be supported by the Administrative core by utilizing the resources provided by the core such as statistical support and teleconferencing.

VIII. Plans for the Next Year

During the remainder of the current funding year (ending 5/31/2014), we plan to continue data

collection and make significant progress on Aim 1 of the current project. We plan to have 100 subjects collected by funding year-end from all age groups and subject groups. We also hope to continue to analyze and publish data resulting from CIFASD Phase II, as described above. Aims 2 & 3 rely on larger sample sizes in our contrast subjects and younger subjects and we plan to emphasize data collection in these groups. Aim 4 focuses on the assessment of memory function in children with FASD. We currently have >70 subjects in our AE and CON groups in the older age group and may initiate analyses consistent with this specific aim.

IX. Publications

As a direct result of CIFASD funding of the current project, the following publications were published or are in press in 2013:

In Press

1. Glass, L., Ware, A.L., Crocker, N., Deweese, B.N., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD. (in press 2013). Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by comorbid ADHD. Neuropsychology.
   DOI: 10.1037/a0033994
   PMC Journal – In Process
   http://www.ncbi.nlm.nih.gov/pubmed/24040921

The following papers are currently under review:

1. Ware, A.L., Glass, L., Crocker, N., Deweese, B.N., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD (Submitted 2013). Effects of prenatal alcohol exposure and ADHD on adaptive functioning.
2. Glass, L., Graham, D.M., Deweese, B.N., Jones, K.L., Riley, E.P., and Mattson, S.N. (Submitted 2013). Correspondence of parent report and laboratory measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure.
3. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure.
4. Discriminating behavioral subtypes among children with heavy prenatal alcohol exposure.

X. Posters and Presentations

In 2012, the following posters and presentations were given.

Poster Presentations

Nguyen, T.T., Glass, L., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., & the CIFASD (2013). The clinical utility and specificity of parent reports of executive functions among children with prenatal alcohol exposure. Presented at the National Academy of Neuropsychology 33rd Annual Conference, San Diego, October 2013.

Nguyen, T.T., Glass, L., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., **Mattson**, S.N., & the CIFASD (2013). Clinical utility of the Behavior Rating Inventory of Executive Function in the identification of children with prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Orlando, June 2013.

Glass, L., Graham, D.M., Deweese, B.N., Riley, E.P., and **Mattson**, S.N. (2013). Parent-Reported Inattention and Hyperactivity: Validation Using Laboratory Measures in Alcohol-Exposed Children. Presented at the 25th APS Annual Convention, Washington, D.C., May 2013.

Invited Presentations

Mattson, S.N. (2013). Fetal alcohol spectrum disorders: Is there a neurobehavioral profile? National Academy of Neuropsychology Annual Meeting, San Diego, CA, October 16-19, 2013.

Mattson, S.N. and Ware, A.L. (2013). Conducting Neuroimaging Studies from a Neuropsychologist's Perspective. Collaborative Perspectives on Addition First Annual Meeting. Atlanta, GA, May 3-4, 2013.

XI. Supplements, Training, and Community

For this reporting period (April 1, 2013 - November 11, 2013), there have not been any revisions or supplements associated with this project.

In terms of training and professional development, we have a training component of our project whereby site staff are trained in the use of the materials needed for the project. Professional development is also accomplished through presentations at conferences aimed at a broad professional audience. Three examples of such presentations are:

Mattson, S.N. (2012). FASD or ADHD? Can You Tell the Difference? Substance Abuse and Mental Health Services Administration FASD Center for Excellence Field Trainers Update. Anaheim, CA, June 12-13, 2012.

Mattson, S.N. (2012). Fetal Alcohol Spectrum Disorders: Is There a Neurobehavioral Profile? 21st Annual Butters-Kaplan West Coast Neuropsychology Conference, San Diego, CA, March 22-25, 2012.

Mattson, S.N. (2012). Fetal Alcohol Spectrum Disorders: Just Another Case Of ADHD? 2nd Annual Southern California Cognitive Neuroscience Meeting, San Diego, CA, March 2, 2012.

Our results been disseminated to communities of interest via publication in scientific journals, which are publically and freely available as per the NIH public access policy, and through presentations at conferences. Importantly, members of the CIFASD team are regularly invited to participate in conferences that target the lay audience, such as:

Mattson, S.N. (2011). Specificity of the Neurobehavioral Profile of FASD. Fifth International Conference on FASD. Vancouver, BC, March 1, 2013.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
In process at NIHMS	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN. Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921; NIHMSID: 538821.
Complete	O'Brien JW, Norman AL, Fryer SL, Tapert SF, Paulus MP, Jones KL, Riley EP, Mattson SN. Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure. Alcohol Clin Exp Res. 2013 Apr;37(4):644-54. PubMed PMID: 23094678; PubMed Central PMCID: PMC3771541.
Complete	Ware AL, O'Brien JW, Crocker N, Deweese BN, Roesch SC, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, CIFASD. The effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on psychopathology and behavior. Alcohol Clin Exp Res. 2013 Mar;37(3):507-16. PubMed PMID: 22974279; PubMed Central PMCID: PMC3524354.
Complete	Mattson SN, Roesch SC, Glass L, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Adnams CM, Jones KL, Riley EP, CIFASD. Further development of a neurobehavioral profile of fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2013 Mar;37(3):517-28. PubMed PMID: 22974253; PubMed Central PMCID: PMC3524344.
Complete	Norman AL, O'Brien JW, Spadoni AD, Tapert SF, Jones KL, Riley EP, Mattson SN. A functional magnetic resonance imaging study of spatial working memory in children with prenatal alcohol exposure: contribution of familial history of alcohol use disorders. Alcohol Clin Exp Res. 2013 Jan;37(1):132-40. PubMed PMID: 23072431; PubMed Central PMCID: PMC3694801.
Complete	Graham DM, Crocker N, Deweese BN, Roesch SC, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, CIFASD. Prenatal alcohol exposure, attention-deficit/hyperactivity disorder, and sluggish cognitive tempo. Alcohol Clin Exp Res. 2013 Jan;37 Suppl 1:E338-46. PubMed PMID: 22817778; PubMed Central PMCID: PMC3480974.

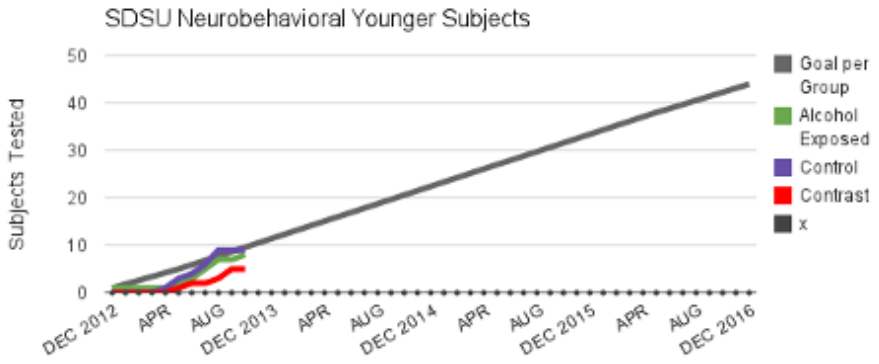
Neurobehavioral Progress 2012-2017				
Site	**Goal Per Group**	**Alcohol-Exposed**	**Control**	**Contrast**
SDSU Old	44	26	24	11
SDSU Young	44	8	12	5
UMN Old	44	28	22	17
UMN Young	44	6	12	2
Emory Old	44	10	14	6
Emory Young	44	13	5	7
USC Old	44	15	10	n/a
	All Groups & Ages	**Alcohol-Exposed**	**Control**	**Contrast**
Tested Total	253	106	99	48
Goal Total	880	308	308	264
Date of Update:	11/4/2013	# Fields	Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific; #s are cumulative.	

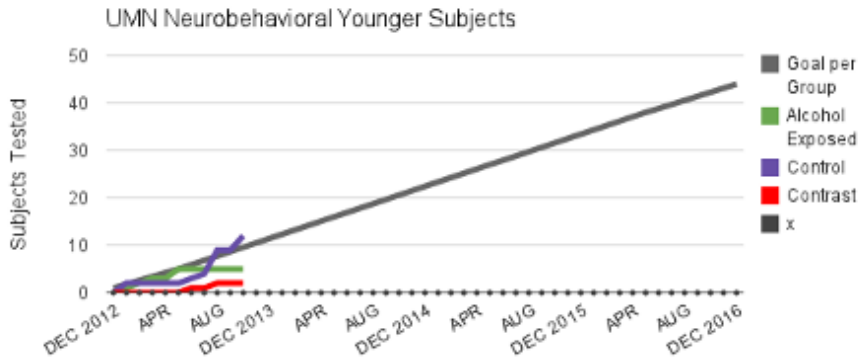
Site		SDSU						UMN						Emory						USC	
Goals (Cumulative)		Old			Young			Old			Young			Old			Young			Old	
Data Collection Subgroups	Thru/By Date	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON
Start YR1	8/10/2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
End YR1	5/31/2013	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
End YR2	5/31/2014	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
End YR3	5/31/2015	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
End YR4	5/31/2016	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
Data Collection End YR5	12/1/2016	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44

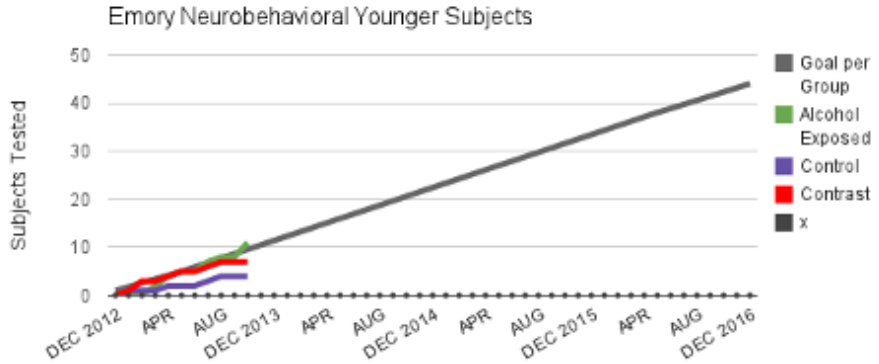
Total group goals across all sites for the older age study = 176 alcohol exposed, 176 control and 132 contrast for a total of 484.
Total group goals across all sites for the younger age study = 132 alcohol exposed, 132 control and 132 contrast for a total of 396. Both studies total = 880.

Explanation of Data Collection Goal Modification: Original proposal of 9 per year (5) per group (20) for a total of 900 (Grant Table 3) was incorrect; numbers should have followed the proposed timeline table in the grant. Overall numbers reduced by 2% to account for shortened first year and budget cuts.

NB Young x in the keys below is to allow for a line to run along the x-axis indicating a hash mark (dot) per month.



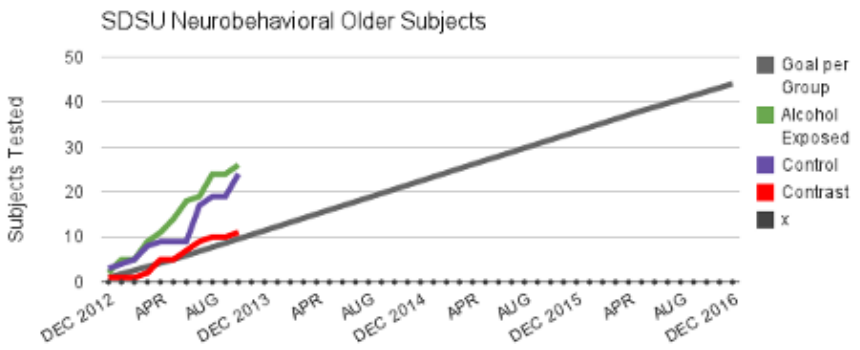


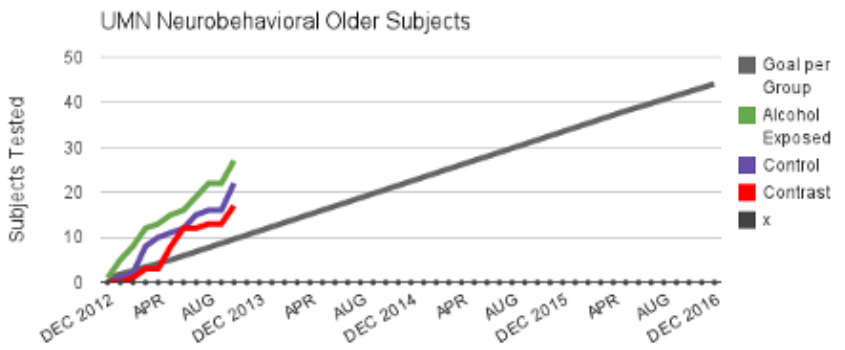


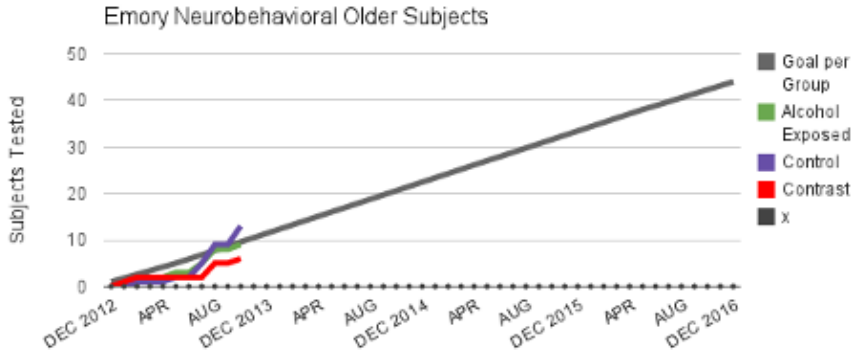
*Jill will complete this each month. Cumulative totals represented. Click Here

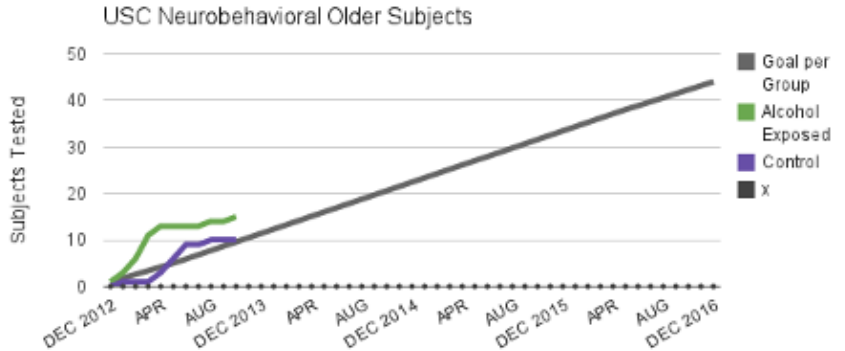
SDSU	Goal per Group	Alcohol Exposed	Control	Contrast	x	UMN	Goal per Group	Alcohol Exposed	Control	Contrast	x	Emory	Goal per Group	Alcohol Exposed	Control	Contrast	x
DEC 2012	1.0	1	0	0	0	DEC 2012	1.0	0	0	0	0	DEC 2012	1.0	0	0	0	0
JAN 2013	1.8	1	0	0	0	JAN 2013	1.8	1	2	0	0	JAN 2013	1.8	0	1	1	0
FEB	2.6	1	0	0	0	FEB	2.6	2	2	0	0	FEB	2.6	0	1	3	0
MAR	3.4	1	0	0	0	MAR	3.4	3	2	0	0	MAR	3.4	2	1	3	0
APR	4.2	1	1	0	0	APR	4.2	3	2	0	0	APR	4.2	4	2	4	0
MAY 2013	5.0	2	3	1	0	MAY 2013	5.0	5	2	0	0	MAY 2013	5.0	5	2	5	0
JUN	5.9	3	4	2	0	JUN	5.9	5	3	1	0	JUN	5.9	5	2	5	0
JUL	6.8	5	6	2	0	JUL	6.8	5	4	1	0	JUL	6.8	7	3	6	0
AUG	7.7	7	9	3	0	AUG	7.7	5	9	2	0	AUG	7.7	8	4	7	0
SEPT	8.7	7	9	5	0	SEPT	8.7	5	9	2	0	SEPT	8.7	8	4	7	0
OCT	9.6	8	9	5	0	OCT	9.6	5	12	2	0	OCT	9.6	11	4	7	0
NOV	10.5				0	NOV	10.5				0	NOV	10.5				0
DEC 2013	11.4				0	DEC 2013	11.4				0	DEC 2013	11.4				0
JAN 2014	12.3				0	JAN 2014	12.3				0	JAN 2014	12.3				0
FEB	13.3				0	FEB	13.3				0	FEB	13.3				0
MAR	14.2				0	MAR	14.2				0	MAR	14.2				0
APR	15.1				0	APR	15.1				0	APR	15.1				0

NB Old x in the keys below is to allow for a line to run along the x-axis indicating a hash mark (dot) per month.









*Jill will complete this each month. Cumulative totals represented. Click Here

SDSU	Goal per Group	Alcohol Exposed	Control	Contrast	x	UMN	Goal per Group	Alcohol Exposed	Control	Contrast	x	Emory	Goal per Group	Alcohol Exposed	Control	Contrast	x	USC	Goal per Group	Alcohol Exposed	Control	x
DEC 2012	1.0	2	3	1	0	DEC 2012	1.0	1	0	0	0	DEC 2012	1.0	0	0	0	0	DEC 2012	1.0	1	0	0
JAN 2013	1.8	5	4	1	0	JAN 2013	1.8	5	1	0	0	JAN 2013	1.8	0	0	1	0	JAN 2013	1.8	3	1	0
FEB	2.6	5	5	1	0	FEB	2.6	8	2	1	0	FEB	2.6	2	1	2	0	FEB	2.6	6	1	0
MAR	3.4	9	8	2	0	MAR	3.4	12	8	3	0	MAR	3.4	2	1	2	0	MAR	3.4	11	1	0
APR	4.2	11	9	5	0	APR	4.2	13	10	3	0	APR	4.2	2	1	2	0	APR	4.2	13	3	0
MAY 2013	5.0	14	9	5	0	MAY 2013	5.0	15	11	8	0	MAY 2013	5.0	3	2	2	0	MAY 2013	5.0	13	6	0
JUN	5.9	18	9	7	0	JUN	5.9	16	12	12	0	JUN	5.9	3	2	2	0	JUN	5.9	13	9	0
JUL	6.8	19	17	9	0	JUL	6.8	19	15	12	0	JUL	6.8	5	5	2	0	JUL	6.8	13	9	0
AUG	7.7	24	19	10	0	AUG	7.7	22	16	13	0	AUG	7.7	8	9	5	0	AUG	7.7	14	10	0
SEPT	8.7	24	19	10	0	SEPT	8.7	22	16	13	0	SEPT	8.7	8	9	5	0	SEPT	8.7	14	10	0
OCT	9.6	26	24	11	0	OCT	9.6	27	22	17	0	OCT	9.6	9	13	6	0	OCT	9.6	15	10	0
NOV	10.5				0	NOV	10.5				0	NOV	10.5				0	NOV	10.5			0
DEC 2013	11.4				0	DEC 2013	11.4				0	DEC 2013	11.4				0	DEC 2013	11.4			0
JAN 2014	12.3				0	JAN 2014	12.3				0	JAN 2014	12.3				0	JAN 2014	12.3			0
FEB	13.3				0	FEB	13.3				0	FEB	13.3				0	FEB	13.3			0
MAR	14.2				0	MAR	14.2				0	MAR	14.2				0	MAR	14.2			0
APR	15.1				0	APR	15.1				0	APR	15.1				0	APR	15.1			0
MAY 2014	16.0				0	MAY 2014	16.0				0	MAY 2014	16.0				0	MAY 2014	16.0			0
JUN	16.9				0	JUN	16.9				0	JUN	16.9				0	JUN	16.9			0

I. Principal Investigator: Elizabeth R. Sowell, Ph.D.

II. Title of Project: Mapping the Brain, the Face and Neurocognitive Function in FASD U01 AA017122

III. Objectives:

In each of 3 Specific Aims, we will test hypotheses regarding: **a.** cross-sectional and longitudinal differences in brain structure (high-resolution T1- and T2-weighted MRI), structural connectivity (diffusion tensor imaging), and functional connectivity (resting state fMRI), and the relationships between or differences among imaging markers in FASD and unexposed children at 4 sites (Los Angeles, San Diego, Atlanta (new), and Minnesota (new)); **b.** relationships among neurocognitive measures and cross-sectional and longitudinal brain "imaging biomarkers;" **c.** relationships between dysmorphology of the face and the brain, and **d.** how dysmorphology in the brains of children associate with findings in animal models.

Aim 1: To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on brain morphology and connectivity using high-resolution T1- and T2-wieghted MRI datasets.
Aim 2. To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on functional connectivity using resting state (rs) fMRI.
Aim 3. To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on structural connectivity using diffusion tensor imaging (DTI).

IV. Methods:

Measures of brain morphometry, structural and functional connectivity and of neural function will be measured with structural MRI (sMRI), DTI and resting state (rs) fMRI.

V. Accomplishments and Results:

Brain Image Acquisition and Neurobehavioral Assessments:
Our "human phantom," and co-investigator, Dr. Katherine Narr, has traveled to all 4 sites (LA, UMN, Emory, and SD). Phantom data from all sites has been uploaded to the servers at the Developmental Cognitive Neuroimaging Laboratory (DCNL) at USC/CHLA, and has been visually inspected, and all acquisition parameters have been confirmed correct as per PING protocols for all 3 platforms (Siemens (UMN, Emory); GE (UCSD), and Phillips (USC/CHLA)).

USC/CHLA: Our CIFASD psychometrician, Genevieve Rodriguez recently left our group in October 2013, but Max Orozco, our new CIFASD psychometrician, received approval for his neurobehavioral pilot subject, and he has been evaluating CIFASD participants over the last 5 months. To date, we have evaluated 37 participants at CHLA neuroimaging

SDSU: A total of 26 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA.

Emory: A total of 31 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA.

UMN: A total of 49 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA.

Facial Imaging and Genetic (saliva) Data Collection at CHLA: We hosted Ken Jones at our site in July 2013 where he did the dysmorphology exam on 21 of our participants. We also collected saliva for genetic analyses on all of our CIFASD participants.

Brain Image Analyses (ongoing from CIFASD II):

We continue to analyze brain image data from CIFASD II, and have begun some analyses on CIFASD III data. We were awarded an F32 training grant to capitalize on the already funded CIFASD III project (Uban PI: 1F32AA022561-01). Below we describe some of our ongoing projects from longitudinal CIFASD data.

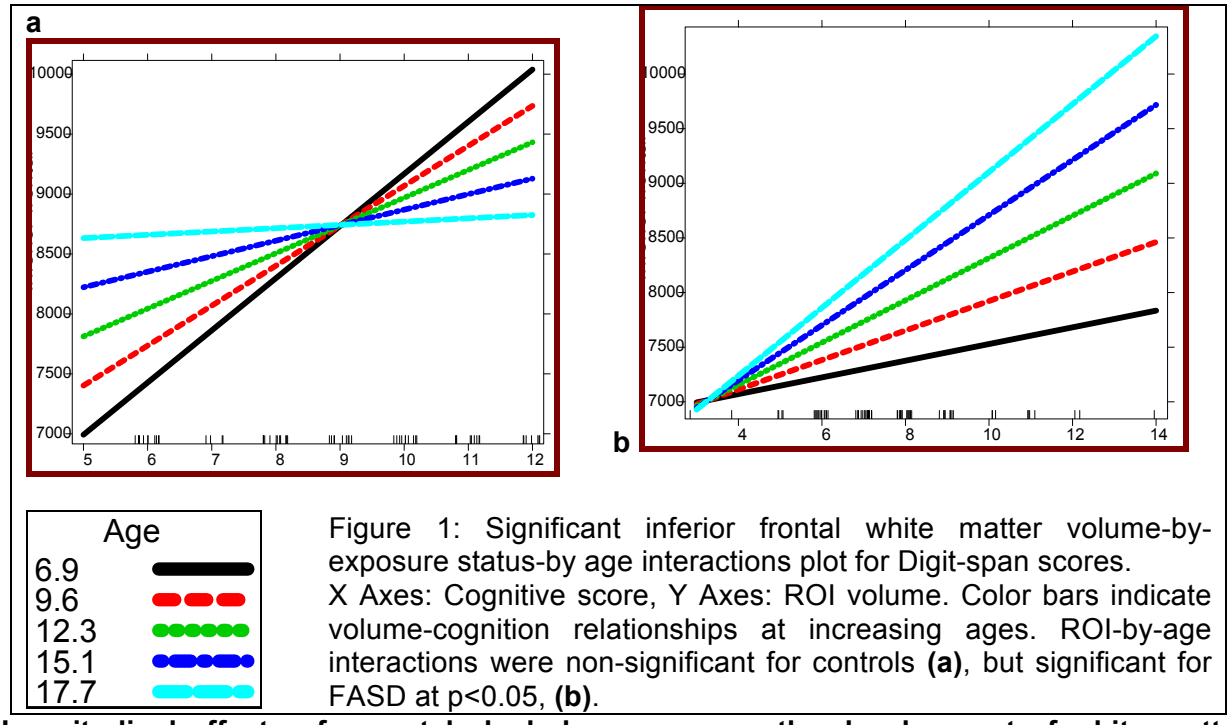


Figure 1: Significant inferior frontal white matter volume-by-exposure status-by age interactions plot for Digit-span scores.
X Axes: Cognitive score, Y Axes: ROI volume. Color bars indicate volume-cognition relationships at increasing ages. ROI-by-age interactions were non-significant for controls **(a)**, but significant for FASD at $p<0.05$, **(b)**.

Longitudinal effects of prenatal alcohol exposure on the development of white matter volume and executive function: In this study, we predicted that the rate of white matter volumetric development would be atypical in children with Fetal Alcohol Spectrum Disorders (FASD) when compared to typically developing children, and the rate of change in cognitive function would relate to differential white matter development between groups. All participants were recruited at UCLA. Data were available for 103 subjects [longitudinal, n= 41 (25 FASD)] and [cross-sectional, n=62 subjects (24 FASD)]. Groups were age-matched. Participants received a structural scan and an executive function (EF) battery (Trails A and B, Digit Span from WISC IV, CVLT-C). Using white matter volumes measured bilaterally for frontal and parietal regions and the corpus callosum, change was predicted by modeling the effects of age, intracranial volume, sex, and interactions among exposure status, EF measures, and age. While both groups showed regional increases in white matter volumes and improvement in cognitive performance over time, there were significant effects of exposure status, on age-related white matter increases for EF measures **(Figure 1).** Specifically, those with FASD consistently showed a positive relationship between cognitive function and white matter volume with age, while no such relationships were seen in controls. Results show for the first time that regional increase in white matter volume over time is differentially related to cognitive change between FASD and typically developing children. These novel results of significant positive

effect on cognitive function with increasing white matter volume suggests that better cognitive outcomes could be possible for FASD subjects through interventions targeting better health outcomes through education and/or other environmental factors.

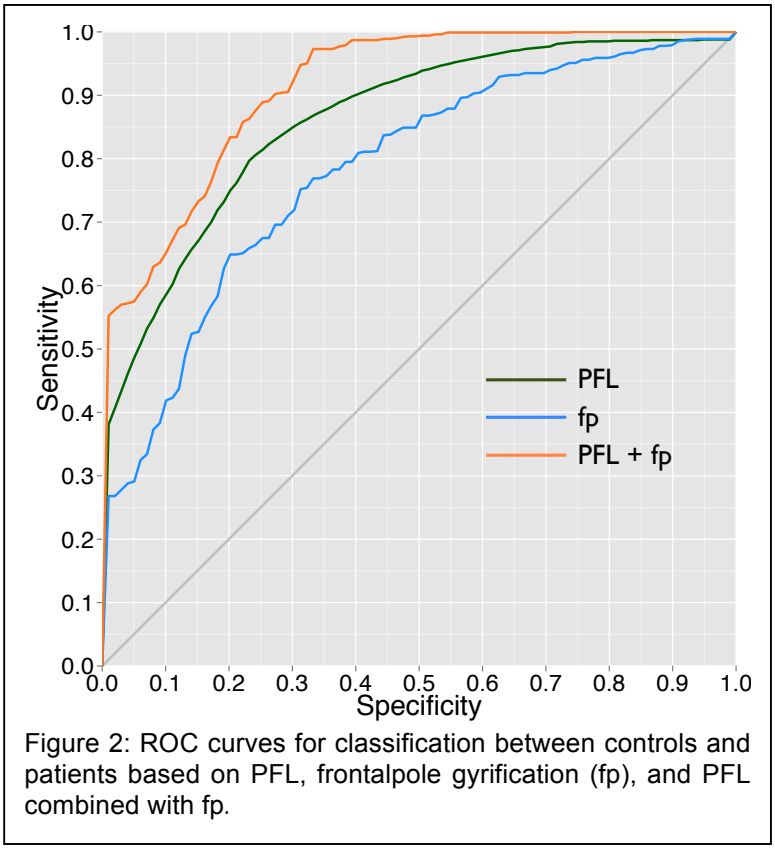


Figure 2: ROC curves for classification between controls and patients based on PFL, frontalpole gyrification (fp), and PFL combined with fp.

Improved classification of diagnosis using brain and face:

Following up on our results presented for a platform presentation at the 19th Annual Meeting of the Organization for Human Brain Mapping in June 2013, we investigated the possibility of combining expert-delineated facial features identified by a dysmorphologist, and automatically computed brain features from the cortex for classifying healthy unexposed controls and diagnose patients. Specifically, we performed classification between controls and patients using 3 different features, i) mean gyrification (brain) of frontalpole (fp), ii) PFL (face), and iii) combined gyrification (brain) and PFL (face). Logistic regression was performed on the 3 features and the classification test was randomly repeated for several times using bootstrapping. **Fig. 2** shows the ROC (receiver operating characteristic) curves for the 3 cases. It is observed that combing PFL (face), and the fp gyrification (brain) yields a better sensitivity (AUC=0.91) compared to either PFL (AUC=0.86) or gyrification (AUC=0.78) alone. Here AUC stands for Area under the curve.

Although, this is a preliminary result, we observe that integrating heterogeneous attributes such as facial landmarks and cortical features, leads to an improvement in classification performance. This supports the notion that both facial and cortical features need to be analyzed in a combined manner to refine the sensitivity of diagnosis in FASD.

Effects of *in utero* alcohol exposure on adolescent brain connectivity (F32 NRSA 1F32AA022561-01, Uban PI): This proposal aims to use a multimodal imaging approach to assess sex differences in the effects of PAE on brain connectivity. In addition, the proposed research project also will assess hormone function in PAE boys and girls, and hormones will be integrated with measures of structural and functional brain connectivity. The proposed findings have implications for cognitive, behavioral and mental health problems that occur at a high prevalence among individuals with an FASD. Results from the proposed studies will help identify sexually dimorphic effects of PAE on underlying neural networks in order to inform future intervention and treatment strategies targeted to a specific sex and specific impairments in neural networks. Since funded, Dr. Uban has collected saliva samples from 22 CIFASD participants at CHLA, and has provided all materials needed for hormone saliva collection for all

participating sites. Our colleagues at UMN have obtained IRB approval to collect saliva for hormones, and are currently collecting this data. San Diego and Emory are in the process of obtaining IRB.

VI. Discussion:

The brain imaging project has been very productive in the last year since the renewal of CIFASD was funded. Data collection has been more rapid than expected, and all sites are meeting their obligations for numbers of subjects imaged. Our human phantom, Dr. Katherine Narr, has either been to, or is scheduled to visit all 4 sites for imaging and protocol inspection. Finally, we continue our publication productivity in the last period of the CIFASD 2 project (see publications listed below) and have published some landmark papers in high profile neuroscience journals (e.g., Lebel et al, J Neuro 2012). We continue to analyze data collected in CIFASD II, and are excited about the new trainee project funded by NIAAA to capitalize on the rich data already collected, and the new data to be collected in CIFASD III.

VII. Integration with Aims of the Consortium and Other Projects:

This project is strongly integrated with the rest of CIFASD in several ways. On the site level, four sites are participating in this component. On the project level, subjects tested for Phase III will be assessed as part of the 3D facial imaging (T. Foroud) and neurobehavioral project (S. Mattson) projects as well as the genetics developmental project (T. Foroud) and data from all projects will be analyzed. Our project also integrates with the Dysmorphology core (K. Jones), the Informatics core (W. Barnett), and the Administrative core (E. Riley). All children will receive a standardized dysmorphology examination, which will provide diagnostic information, aid subject classification, and provide data for the aims of the Dysmorphology core. Thus far, our integration with other projects has included evaluating relationships between the dysmorphology core, and the neurobehavioral project and various brain imaging data modalities. These projects will continue as we further exploit data collected in CIFASD II, and will follow new directions as we near completion of time 1 evaluations from CIFASD III.

VIII. Plans for the Next Year:

We will continue data collection for CIFASD III. Data analyses and manuscript preparation from CIFASD II will continue. We will schedule visits from the 3D facial imaging project, and for dysmorphology from Dr. Ken Jones on new participants since his last visit in July 2012.

IX. Publications

Graham, D. M., Crocker, N., Deweese, B. N., Roesch, S. C., Coles, C. D., Kable, J. A., . . . Cifasd. (2013). Prenatal Alcohol Exposure, Attention-Deficit/Hyperactivity Disorder, and Sluggish Cognitive Tempo. *Alcoholism-Clinical and Experimental Research, 37*, E338-E346. doi: 10.1111/j.1530-0277.2012.01886.x

Lebel, C., Mattson, S. N., Riley, E. P., Jones, K. L., Adnams, C. M., May, P. A., . . . Sowell, E. R. (2012). A Longitudinal Study of the Long-Term Consequences of Drinking during Pregnancy: Heavy In Utero Alcohol Exposure Disrupts the Normal Processes of Brain Development. *Journal of Neuroscience, 32*(44), 15243-15251. doi: 10.1523/jneurosci.1161-12.2012

Mattson, S. N., Roesch, S. C., Glass, L., Deweese, B. N., Coles, C. D., Kable, J. A., . . . Cifasd. (2013). Further Development of a Neurobehavioral Profile of Fetal Alcohol Spectrum Disorders. *Alcoholism-Clinical and Experimental Research, 37*(3), 517-528. doi: 10.1111/j.1530-0277.2012.01952.x

Nguyen, T., Glass, L., Coles, C., Julie, K., May, P., Sowell, E., . . . Mattson, S. (2013). The Clinical Utility and Specificity of Parent Reports of Executive Functions among Children with Prenatal Alcohol Exposure. *Archives of Clinical Neuropsychology, 28*(6), 617-617.

Ware, A. L., O'Brien, J. W., Crocker, N., Deweese, B. N., Roesch, S. C., Coles, C. D., . . . Cifasd. (2013). The Effects of Prenatal Alcohol Exposure and Attention-Deficit/Hyperactivity Disorder on Psychopathology and Behavior. *Alcoholism-Clinical and Experimental Research, 37*(3), 507-516. doi: 10.1111/j.1530-0277.2012.01953.x

Submitted Manuscripts:

Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER. " Prenatal exposure to alcohol alters developmental trajectories for visuo-spatial attention: A multisite longitudinal fMRI study"(under review at *Cerebral Cortex*).

Diana M Graham, MA; Benjamin N Deweese, BA; Scott C Roesch, PhD; Claire D Coles, PhD; Julie A Kable, PhD; Philip A May, PhD; Wendy O Kalberg, PhD; Elizabeth R Sowell, PhD; Kenneth Lyons Jones, MD; Edward P Riley, PhD; Sarah Mattson, Ph.D. Discriminating behavioral subgroups among children with heavy prenatal alcohol exposure".

In Preparation:

Gautam P, Nuñez SC, Narr KL, Mattson SN, Riley EP, Kan EC, Sowell ER. "Longitudinal effects of prenatal alcohol exposure on the development of white matter volume and executive function. Manuscript in preparation

S. H. Joshi, K. L. Narr, E. Kan, R. P. Woods, A. W. Toga, S.N. Mattson, E.P. Riley, K.L. Jones, C.M. Adnams, P.A. May, M.J. O'Connor, and E. Sowell (2013). Reduced Gyrification in Fetal Alcohol Syndrome. Manuscript in preparation

X. Posters and Presentations:

Nguyen, T. T., Glass, L., Coles, C. D., Kable, J. A., May, P. A., Kalberg, W. O., . . . Cifasd. (2013). CLINICAL UTILITY OF THE BEHAVIOR RATING INVENTORY OF EXECUTIVE FUNCTION IN THE IDENTIFICATION OF CHILDREN WITH PRENATAL ALCOHOL EXPOSURE. *Alcoholism-Clinical and Experimental Research, 37*, 44A-44A.

Sowell, E. (2013). Imaging Human Brain Development: Impact of Alcohol. *Biological Psychiatry, 73*(9), 250S-250S.

Uban, K. A., Herting, M. M., & Sowell, E. R. (2013). SEXUALLY DIMORPHIC ALTERATIONS IN STRUCTURAL CONNECTIVITY IN YOUTH WITH A FETAL ALCOHOL SPECTRUM DISORDER. *Alcoholism-Clinical and Experimental Research, 37*, 102A-102A.

S. H. Joshi, K. L. Narr, E. Kan, R. P. Woods, A. W. Toga, S.N. Mattson, E.P. Riley, K.L. Jones, C.M. Adnams, P.A. May, M.J. O'Connor, and E. Sowell (2013). Abnormal Patterns of Gyrification in Fetal Alcohol Syndrome. 19th Annual Meeting of the Organization for Human Brain Mapping, Seattle, Washington.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Sowell ER, Mattson SN, Kan E, Thompson PM, Riley EP, Toga AW. Abnormal cortical thickness and brain-behavior correlation patterns in individuals with heavy prenatal alcohol exposure. Cereb Cortex. 2008 Jan;18(1):136-44. PubMed PMID: 17443018; PubMed Central PMCID: PMC2770438.
Complete	Mattson SN, Foroud T, Sowell ER, Jones KL, Coles CD, Fagerlund A, Autti-Rämö I, May PA, Adnams CM, Konovalova V, Wetherill L, Arenson AD, Barnett WK, Riley EP, CIFASD. Collaborative initiative on fetal alcohol spectrum disorders: methodology of clinical projects. Alcohol. 2010 Nov-Dec;44(7-8):635-41. PubMed PMID: 20036488; PubMed Central PMCID: PMC2888656.
Complete	Sowell ER, Leow AD, Bookheimer SY, Smith LM, O'Connor MJ, Kan E, Rosso C, Houston S, Dinov ID, Thompson PM. Differentiating prenatal exposure to methamphetamine and alcohol versus alcohol and not methamphetamine using tensor-based brain morphometry and discriminant analysis. J Neurosci. 2010 Mar 17;30(11):3876-85. PubMed PMID: 20237258; PubMed Central PMCID: PMC2847574.
Complete	Nuñez SC, Dapretto M, Katzir T, Starr A, Bramen J, Kan E, Bookheimer S, Sowell ER. fMRI of syntactic processing in typically developing children: structural correlates in the inferior frontal gyrus. Dev Cogn Neurosci. 2011 Jul;1(3):313-23. PubMed PMID: 21743820; PubMed Central PMCID: PMC3129989.
Complete	Yang Y, Phillips OR, Kan E, Sulik KK, Mattson SN, Riley EP, Jones KL, Adnams CM, May PA, O'Connor MJ, Narr KL, Sowell ER. Callosal thickness reductions relate to facial dysmorphology in fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2012 May;36(5):798-806. PubMed PMID: 22150665; PubMed Central PMCID: PMC3309126.
Complete	Colby JB, Soderberg L, Lebel C, Dinov ID, Thompson PM, Sowell ER. Along-tract statistics allow for enhanced tractography analysis. Neuroimage. 2012 Feb 15;59(4):3227-42. PubMed PMID: 22094644; PubMed Central PMCID: PMC3288584.
Complete	Sowell ER, Johnson A, Kan E, Lu LH, Van Horn JD, Toga AW, O'Connor MJ, Bookheimer SY. Mapping white matter integrity and neurobehavioral correlates in children with fetal alcohol spectrum disorders. J Neurosci. 2008 Feb 6;28(6):1313-9. PubMed PMID: 18256251; PubMed Central PMCID: PMC3567846.

Complete	Lebel C, Mattson SN, Riley EP, Jones KL, Adnams CM, May PA, Bookheimer SY, O'Connor MJ, Narr KL, Kan E, Abaryan Z, Sowell ER. A longitudinal study of the long-term consequences of drinking during pregnancy: heavy in utero alcohol exposure disrupts the normal processes of brain development. J Neurosci. 2012 Oct 31;32(44):15243-51. PubMed PMID: 23115162; PubMed Central PMCID: PMC3515671.
Complete	Colby JB, Smith L, O'Connor MJ, Bookheimer SY, Van Horn JD, Sowell ER. White matter microstructural alterations in children with prenatal methamphetamine/polydrug exposure. Psychiatry Res. 2012 Nov 30;204(2-3):140-8. PubMed PMID: 23149028; PubMed Central PMCID: PMC3634917.
Complete	Roussotte FF, Sulik KK, Mattson SN, Riley EP, Jones KL, Adnams CM, May PA, O'Connor MJ, Narr KL, Sowell ER. Regional brain volume reductions relate to facial dysmorphology and neurocognitive function in fetal alcohol spectrum disorders. Hum Brain Mapp. 2012 Apr;33(4):920-37. PubMed PMID: 21416562; PubMed Central PMCID: PMC3812802.

CIFASD 2013 mid-period progress report for U01-AA021651 and its Supplement

October 24, 2013

I. Principal Investigator: Kathleen K. Sulik

II. Title of Project: Craniofacial and CNS pathology in a mouse FASD model

III. Objectives: The primary objective of this work is to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. This work naturally builds on our CIFASD-supported basic research to date and continues to address the need for a more complete understanding of the spectrum and exposure stage-dependency of abnormalities caused by prenatal alcohol exposure. **Our overall hypothesis is that alcohol induces structural abnormalities of the brain and face of mice that are consistent with and informative for those in human FASD.** The 3 original Specific Aims for this grant are as follows: Aim 1. to examine correlations between the dysmorphology of the brain and the face that result from alcohol exposure at specific early stages of prenatal development in mice; Aim 2. to delineate early prenatal alcohol exposure-induced cerebral cortical thickness alterations and associated fiber tract and structural connectivity changes in postnatal mice; and Aim 3. to further define the histopathology and genesis of early prenatal alcohol exposure-induced regional brain dysmorphology.

IV. Methods: Methods: Correlative brain and face dysmorphology studies are being conducted on fetal mice using high-resolution Magnetic Resonance Imaging (MRI) and dense surface modeling (DSM). Fetal animals are being collected from groups of control dams as well as from those that had been administered alcohol acutely on days 10 or 11.5 of pregnancy. Optical projection tomography (OPT) techniques are also being developed for additional fetal brain/face assessments. Additionally, the brains of postnatal mice whose dams had been treated acutely on day 7 of pregnancy are being examined using atlas-based MRI & DTI analysis techniques to assess regional brain volumes, cortical thickness changes, and fiber tract alterations. Magnesium enhanced MRI (MEMRI) techniques are being developed and applied to enhance our understanding of structural/functional relationships. Histological methodologies are also being employed for analysis of regions of interest in pre- and postnatal brains. In addition to these structural analysis techniques, mutant mice are being used to examine gene/environment interactions and a high potency cannabinoid, CP-55940, is being used for a co-teratogen exposure study.

V. Accomplishments and Results: Overall, our progress in the past 6 months has been steady and substantial. During this time our CIFASD-funded publications have included 1 full paper, and 4 abstracts. Two additional papers are being finalized and should be for submitted in November, 2013. During this reporting period, Dr. Sulik gave an invited presentation at an FASD legal issues conference in Edmonton, Alberta, presenting her lab's CIFASD-supported research findings. Importantly, during this time period, Dr. Shonagh O'Leary-Moore left academia and Dr. Rob Lipinski took a faculty position at the University of Wisconsin. In adjusting to this change in our lab personnel, and in order to continue to productively address our primary research objective, Dr. Eric Fish (research associate), Dr. Lindsay Wieczorek (postdoctoral fellow), and Dr. Scott Parnell (assistant Professor) have joined our CIFASD effort.

To date, MRI scanning for stage of exposure-dependent brain and facial dysmorphology analyses of GD 17 fetuses has been completed for 6 litters from each of two maternal alcohol-exposure times (GD10 and 11.5), as well as for 6 control litters. Although in the past our work

has relied heavily on manual segmentation of fetal brains, we have been working with Dr. Hammond and his colleagues to apply atlas-based regional brain segmentations for brain shape and volume analyses. This provides for a substantial reduction in image processing time and in consequent financial savings.

Following up on our previous study employing MRI and DSM to study and compare brain and facial dysmorphology resulting from GD7 or 8.5 alcohol exposure, and in keeping with the consortium's interest in genetically-based susceptibilities (gene/enveironment interactions), we have conducted and reported a study illustrating that Shh and Gli2 mutation in mice greatly increases GD7 alcohol exposure-induced teratogenesis (Figure 1). Additionally, we have initiated a similar GD8.5 study, testing the hypothesis that developmental stage-dependent alterations in the Shh signaling pathway can yield opposing phenotypes; i.e. a face and brain that are too narrow from GD7 alcohol exposure versus too wide from GD 8.5 alcohol exposure. For this study, we plan to use a new technique, optical projection tomography, to aid in assessing face and brain changes. To further illustrate that insult to the Shh pathway underlies alcohol-induced teratogenesis, a study employing the Smoothened agonist, SAG, is being initiated. This study will test the hypothesis that by upregulating Smoothened, alcohol teratogenesis can be diminished.

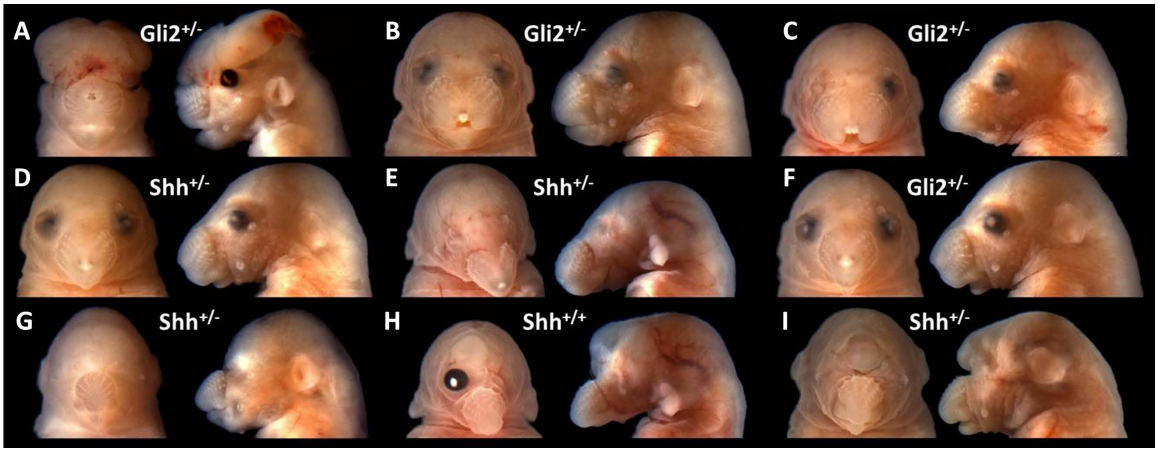


Figure 1. Subpopulation of GD17 mouse fetuses exhibiting severe craniofacial phenotypes. Shown are 9 fetuses with phenotypes not typically observed in wildtype C57BL/6J mice exposed to the employed alcohol exposure paradigm (A-I). Single allele mutations in Shh or Gli2 were detected in 8 of 9 fetuses in this severely affected subpopulation. In addition to varying degrees of upper midfacial deficiency, other notable defects included exencephaly (A), iridial coloboma and microphthalmia (A-D), apparent anophthalmia (E, G, I), agnathia (E), micrognathia (A-D, F-I), and proboscis (I). Median cleft lip was also observed (B). Within this subpopulation, fetuses were assigned dysmorphology scores as follows: 2 (A), 3 (B-F), 4 (G-I).

During this reporting period, we have also initiated a study directed toward examining potential teratogenic interactions between alcohol and high potency cannabinoids. The rationale for this work lies in the premise that alcohol and cannabinoids are commonly concurrently used and that this co-drug exposure (environment/environment interaction) will yield more severe teratogenic outcomes than each agent alone. This work also rests on the fact that new "designer" cannabinoids are extremely potent and little teratogenicity data exists for them. Pilot data collected to date employing administration of the synthetic cannabinoid, CP-55,940, on GD 7 or 8 in mice illustrate induction of craniofacial malformations including anophthalmia, microphthalmia, iridial coloboma, exencephaly, midfacial deficiencies consistent with

holoprosencephaly, and median facial clefting. These defects are consistent with those that result from alcohol exposure at these same time periods.

Finally, progress toward identification of fiber tract changes following GD 7 alcohol exposure includes completion of the MR scanning for 48 adult mice, with data in the pipeline for analyses. Importantly, the structural analyses are being coupled with functional endpoints. To further our understanding of structure/function relationships we are hoping to apply Magnesium Enhanced MRI (MEMRI) techniques to our model system. Pilot studies to date on 5 control and 5 prenatal alcohol-exposed adult mice are promising, with stress resulting in signal in expected brain regions.

VI. Discussion and Significance: A highlight of our work during this time period is finalization of our manuscript describing the results of our Shh and Gli2 mutant mouse study. This study has provided a springboard for additional gene/environment studies and for more in depth study of the interaction of alcohol with genes in, or affecting the Shh pathway. We are particularly excited to initiate studies employing the Smoothened agonist, SAG, as this agent is expected to ameliorate alcohol's teratogenicity. As further discussed in the attached supplement progress report, we are also excited to extend our current work by beginning to look at potential teratogenic interactions between alcohol and high potency cannabinoids. We feel that this work is timely, important for the FASD field, and in keeping with our primary objective of making clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. In addition to these gene/environment and environment/environment studies, we have begun to correlate our structural findings with functional endpoints. Importantly, a significant amount of the work conducted during this reporting period has been directed toward establishing new methodologies (MEMRI and OPT) and conducting pilot studies that provide the foundation for our continuing FASD research.

VII. Interrelation with the Aims of the Consortium and Other Projects: Our correlative brain/face studies complement those of Dr. Faroud's group and we will continue to work closely with Dr. Peter Hammond and colleagues on the analyses of facial dysmorphology induced by stage-dependent alcohol insult. Our DTI studies complement those of Dr. Sowell and Dr. Wozniak. And, our studies assessing genetically modified mice and their susceptibility to alcohol teratogenesis complement and extend the work of Dr. Johann Eberhart.

VIII. Plans for the next 6 months:

1. Submit 2 manuscripts for publication
2. Work with Mike Suttie to complete data processing and analyses for GD 10 and 11.5 exposures.
3. Follow up on gene/environment studies, examining Shh and Gli mutant mice following alcohol exposure on GD 8.5 and working with SAG to illustrate the importance of SHH pathway perturbation and to potentially identify a new ameliorative agent.
4. Continue pilot studies looking at the potential of "super" cannabinoids to interact with alcohol as a teratogen
5. Continue pilot MEMRI studies to supplement the MRI and DTI studies in providing structure/function correlates

IX. Publications:

1. Parnell SE, Holloway HT, O'Leary-Moore SK, Dehart DB, Paniaqua B, Oguz I, Budin F, Styner MA, Johnson GA, Sulik KK. Magnetic resonance microscopy-based analyses of

the neuroanatomical effects of gestational day 9 ethanol exposure in mice. Neurotoxicol Teratol. 2013 Sep-Oct;39:77-83. doi: 10.1016/j.ntt.2013.07.009. Epub 2013 Jul 30. PubMed PMID: 23911654; PubMed Central PMCID: PMC3795920.

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Parnell SE, Holloway HT, O'Leary-Moore SK, Dehart DB, Paniaqua B, Oguz I, Budin F, Styner MA, Johnson GA, Sulik KK. Magnetic resonance microscopy-based analyses of the neuroanatomical effects of gestational day 9 ethanol exposure in mice. Neurotoxicol Teratol. 2013 Sep-Oct;39:77-83. PubMed PMID: 23911654; PubMed Central PMCID: PMC3795920.

2. S.E. Parnell; H.T. Holloway; B. Paniagua; I. Oguz; M.A. Styner; G.A. Johnson; K.K. Sulik. Dysmorphogenic effects of chronic gestational ethanol exposure on the prenatal mouse: a magnetic resonance microscopy-based study. **(being finalized for submission to ACER)**
3. Henry W. Keitzman, Joshua L. Everson, Kathleen K. Sulik, Robert J. Lipinski. The teratogenic effects of murine prenatal ethanol exposure are exacerbated by Shh or Gli2 haploinsufficiency. **(being finalized for submission to PLOS 1)**

X. Poster Abstract References and Presentations

1. O'Leary-Moore SK, Budin F, Paniagua B, Oguz I, Johnson GA, Sulik KK. High-resolution neuroimaging reveals a range of corpus callosum insult induced by ethanol on gestational day 7 in mice. Alcohol Clin Exper Res, 37 suppl 2:167A, 2013
2. Kietzman HW, Sulik KK, Lipinski RJ. The effect of Shh and Gli2 heterozygosity in a mouse model of Fetal Alcohol Syndrome. Alcohol Clin Exper Res, 37 suppl 2:167A, 2013
3. Sulik KK. Prenatal alcohol exposure and abnormal brain development: Insights from animal studies. Proceedings of the FASD legal issues consensus development conference, Edmonton, Alberta Canada, September, 2013
4. H.W. Kietzman; K.K. Sulik; R.J. Lipinski, The interaction of *Shh* and *Gli2* mutations with prenatal ethanol exposure in the genesis of fetal alcohol syndrome and holoprosencephaly. DW Smith Workshop on Malformations and Morphogenesis, Mt Tremblant, Canada, August, 2013
5. Marcoita T. Gilbert, Scott E. Parnell, Eric Fish, Lorinda Baker, and Kathleen K. Sulik. Exogenous cannabinoid administration during early gestation results in a high incidence of congenital abnormalities in the mouse. Carolina Collaborative Cannabinoid Conference, Oct, 2013 (submitted)

Book chapters:

1. Sulik KK, Fetal Alcohol Spectrum Disorder: Pathogenesis and Mechanisms, In: Pfefferbaum and Sullivan, Alcohol and the Nervous System 1E (Handbook of Clinical Neurology), Elsevier, 2013 (in press)

XI. Supplements, Training, and Community

Supplement (AA021651-01S1) to Promote Diversity in Health-Related Research
Principal Investigator: Kathleen K. Sulik (mentor of Marcoita Gilbert)

Title of Project: Craniofacial and CNS Pathology in a Mouse FASD Model

Objectives and Goals: This diversity supplement is directed toward promoting the health science training and career of Dr. Marcoita Gilbert. As funded, it supports both research and mentoring/career development activities. The primary objective of the basic research is to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. This work naturally builds on our CIFASD-supported basic research to date and continues to address the need for a more complete understanding of the spectrum and exposure stage-dependency of abnormalities caused by prenatal alcohol exposure. Our overall hypothesis is that alcohol induces structural abnormalities of the brain and face of mice that are consistent with and informative for those in human FASD.

Accomplishments: Activities and progress are outlined as per the original timeline.

March 2013 through May 2013 –
-Responsible Conduct of Research (RCR) training, March 18-19, 2013
-Basic Bioinformatics Tools (BBT) workshop, March 19
-Professional development advisory committee meeting, April 23, 2013;
Committee members in attendance were Dr. Donita Robinson, Dr. Fulton Crews and Dr. Kathy Sulik
-Completed Sulik laboratory Embryology course

June 2013 through August 2013 –
-Attended RSA meeting
-Leadership role in importing and establishing Lrp2 +/- mice for a new study conceptualized while meeting with Dr. Johann Eberhart at the January 2013 CIFASD meeting
- Drs. Allyn Howlett and Scott Parnell were invited to join professional development advisory committee
- Initiated study to extend the laboratory's work with Shh and Gli2 mutant mice (see Kietzman et al 2013) to include examination of GD 8.5 alcohol insult. Worked with Dr. Eric Fish to re-establish the breeding colonies, genotyping and treatment protocols and with Dr. Parnell to implement optical projection tomography (OPT)-based morphological analyses.
- Initiated study directed toward examining potential teratogenic interactions between high potency cannabinoids and alcohol. Worked with Dr. Fish to establish cannabinoid exposure paradigm.
- Participated in Sulik laboratory mouse neurobiology review course.
- Gave lab meeting presentations on cannabinoid teratogenicity and on teratogenic gene/environment interactions

Sept. 2013 through Nov. 2013

- Attended Carolina Collaborative Cannabinoid Conference, Oct 2013

Discussion: Dr. Gilbert continues to make substantial progress in becoming well-versed in basic science and clinical issues related to FASD. This is providing a foundation for her current and future research. She has also successfully transitioned from her graduate research avian model to employing a mouse model system. Regarding Dr. Gilbert's research to date, in addition to initiating studies outlined in her proposal, she has also taken the lead in planning and beginning to execute other studies of interest to her and that are in keeping with the CFASD goals. As previously noted, a study directed toward examining alcohol-exposed LRP2 knockout mice was conceptualized at the January 2013 CIFASD meeting following data presentation and discussion with Dr. Johann Eberhart. For this, Dr. Gilbert took the lead in acquiring LRP 2 knockout mice from Dr. Willnow in Germany. Unfortunately, the colony has not been productive and we are currently considering dropping this project. The Shh/Gli2 study that Dr. Gilbert is conducting promises to provide further support for the involvement of interference by alcohol with the Shh signaling pathway. It also promises to aid our understanding of the developmental stage-dependent genesis of opposing facial/brain phenotypes (too narrow versus too wide). For this work, Dr. Gilbert is extending her technical expertise, employing genotyping protocols and optical projection tomography (OPT)-based morphological analyses. The cannabinoid/alcohol interaction study that Dr. Gilbert has initiated wonderfully combines her graduate cannabinoid research expertise with what may be a very important clinical FASD issue. The later being consideration of the combined adverse effect of high potency cannabinoids and alcohol on prenatal development. Pilot studies conducted in C57Bl/6J mice to date are promising, with comparable facial and ocular abnormalities resulting from GD 7 or 8.5 cannabinoid exposure as result from alcohol by itself at these same time periods. Overall, Dr. Gilbert has demonstrated the initiative, enthusiasm and drive that exemplify a successful scientist.

Interrelation with the Aims of the Consortium and Other Projects: The gene/ environment studies being conducted complement those of the Eberhart and Faroud groups.

Plans for the next 6 months

Dec. 2013 through Feb. 2014
- Professional development advisory committee meeting #2
- Continue participating in the laboratory's mouse neurobiology review course
- Collect fetuses from Shh and Gli mutant alcohol exposure studies
- Collect GD7 & 8 cannabinoid-exposed fetuses and analyze data

March 2014 through May 2014
- Attend CIFASD meeting in DC
- Report results of mutant mouse studies
- Initiate cannabinoid/alcohol co-exposure studies
- participate in grantsmanship course

Submitted Abstract: Marcoita T. Gilbert, Scott E. Parnell, Eric Fish, Lorinda Baker, and Kathleen K. Sulik, Exogenous cannabinoid administration during early gestation results in a high incidence of congenital abnormalities in the mouse. Carolina Collaborative Cannabinoid Conference, Oct, 2013

Training and Community

Dr. Sulik participated in an FASD legal issues consensus development conference in Edmonton, Alberta Canada, September, 2013. A newspaper article regarding the meeting can be found at: http://www.edmontonjournal.com/health/Edmonton+conference+calls+changes+justice+system

+protect/8941032/story.html The transcript of Dr. Sulik's presentation, including related questions and discussions, is in the process of being edited for publication. In addition a video of her presentation will be posted to the Alberta Institute of Health Economics website.

On October 12, 2013, Dr. Sulik gave a presentation on FASD for the Together for Resilient Youth Parent Conference In Durham NC (see event announcement below).